Vol. 36 No. 3 2023

Print ISSN: 0971-8184
Online ISSN: 0976-1926

Indian Journal of Plant Genetic Resources

An International Journal for Conservation and Use of Plant Diversity

Indian Society of Plant Genetic Resources
New Delhi, India

INDIAN SOCIETY OF PLANT GENETIC RESOURCES

(Registration No. S/18336)

The Society was founded in 1987 with the following objectives:

- To serve and promote the scientific cause and to advance academic interest in the field of plant genetic resources.
- To disseminate knowledge relating to various aspects on plant genetic resources.
- To provide a forum for organizing symposia/conferences with a view to develop close relationship among the scientists engaged and interested in plant genetic resources activities.

Indian Journal of Plant Genetic Resources, the official publication of the Society, is published thrice in a year. The contribution to the journal, except for invited papers, is open to the members of the society only.
Membership to the society is open to all the individuals/institutions interested in various aspects of plant genetic resources. The membership fee is as follows:

Membership*	Inland		Foreign	
Life Member	Rs.	5,000	US$	1,500
Annual Member	Rs.	1,000	US$	100
Institutional (Annual)	Rs.	20,000	US$	1,000
Institutional (5 Years)	Rs.	90,000	US$	4,500
Institutional (10 Years)	Rs.	1,75,000	US$	8,500

Issues of the journal published earlier are also available.

Limited space is available for advertisement of interest to botanists/geneticists/plant breeders and all those concerned with plant genetic resources.

CORRESPONDENCE relating to membership, advertisement and other related matters should be addressed to the General Secretary, Indian Society of Plant Genetic Resources, ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110 012, India (E-mail: ispgr2015@gmail.com).

COMMUNICATIONS regarding the publication of research papers should be addressed to
Editor-in-Chief, Indian Journal of Plant Genetic Resources, Indian Society of Plant Genetic Resources, C/o ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110 012, India
(E-mail: ispgr2015@gmail.com).

ISSN: 0971-8184
Online ISSN: 0976-1926

Indian Journal of Plant Genetic Resources

Vol. 36 No. 3 2023

INDIAN SOCIETY OF PLANT GENETIC RESOURCES
NBPGR CAMPUS, NEW DELHI-110012, INDIA
Web: ispgr.nbpgr.ernet.in

INDIAN SOCIETY OF PLANT GENETIC RESOURCES

Patrons	MS Swaminathan, RS Paroda, Mangala Rai
Honorary Fellows	MS Swaminathan, GS Khush, S Rajaram, RS Paroda, Mangala Rai, Emile Frison, RB Singh, SK Datta, HY Mohan Ram, KS Gill, SK Vasal, Kamal Bawa, PL Gautam

EXECUTIVE COUNCIL (2022-24)

President	RS Paroda (New Delhi)		
Vice Presidents	RK Tyagi (New Delhi); Jai Chand Rana (New Delhi)		
General Secretary	Anuradha Agrawal (New Delhi)		
Joint Secretary	Manjusha Verma (New Delhi)		
Treasurer	Sanjeev Kumar Singh (New Delhi) (Up to October 2, 2023), Kuldeep Tripathi (w.e.f. 24.10.2023)		
Ex Officio Member	GP Singh, Director, ICAR-NBPGR		
Councillors	North Zone:	Monika Singh (New Delhi)	Kuldeep Tripathi (New Delhi)
	East Zone:	Mohan Lal (Jorhat)	Veerendra Kumar Verma (Umiam)
	West Zone:	Sandip K. Bera (Junagadh)	Anil Patidar (Jaisalmer)
	South Zone:	N. Sivaraj (Hyderabad)	Rose Mary Francies (Thrissur)
	Central Zone:	Shailesh Tiwari (Varanasi)	Surendra Kumar Barpete (Madhya Pradesh)

CONTENTS

1. **Study on Genetic Variability and Correlation of Yield Traits in the F3 Population Derived from Wild Introgression Lines of Swarna/*Oryza nivara*** **341**
Kavitha Beerelli, Divya Balakrishnan, Jyothi Badri, Senguttuvel P, Gireesh C, Ladhalakshmi D, Tuti MD, Padmavathi G, Subba Rao LV and Sarla Neelamraju

2. **Characterization and Evaluation of Faba Bean (*Vicia faba* L.) Germplasm Collected from Kashmir, India** **346**
Sheikh M. Sultan, Susheel K. Raina, Suheel Ahmad and Sheeraz S. Bhat

3. **Genetics of Yield and Fruit Quality Traits in Bitter Gourd (*Momordica charantia* L.)** **356**
Vinay N. D, Asima Aamin, Tusar K. Behera, Anilabh D. Munshi, Gograj S. Jat, Shyam S. Dey and Ranjith K. Ellur

4. **Agro-morphological Characterization and Digitization of Job's Tears (*Coix lacryma-jobi* L.) Germplasm: A Minor Cereal Crop of Northeastern India** **362**
Laxmisha KM, Kailash C. Bhatt, Mamta Arya, Rakesh Bhardwaj, Dinesh P. Semwal and Veena Gupta

5. **Screening of wild and cultivated Vigna accessions for resistance to bruchid (*Callosobruchus maculatus* F)** **371**
Ragul Subramaniyan and Manivannan Narayana

6. **Biophysical and Morphological Basis of Resistance against Linseed (*Linum usitatissimum* L.) Bud fly (*Dasyneura lini* Barnes)** **382**
Vinay Kumar, Gautham S, Kavita Gupta, V.K. Biradar, Vikender Kaur, Beena Nair and Ajay Kumar

7. **Elucidating Diversity among Mango (*Mangifera indica* L.) Hybrids based on Morphological Characters using DUS Guidelines** **387**
Gulshan Kumar, Manish Srivastav, Sreekanth H.S., Jai Prakash, Sanjay K. Singh and Vinod

8. **Development of Cryoconservation Protocol for In-vitro Shoot Bases of *Allium ampeloprasum* L. for Long-term Conservation** **396**
Ravi Gowthami, Subhash Chander, Ruchira Pandey, Muthusamy Shankar and Anuradha Agrawal

9. **Phenotypic Characterization of Crop Genetic Resources of AA-genome Species in Rice (*Oryza spp*.)** **402**
Aswin Mahendran, Mahesh C. Yadav, Gopala Krishnan S, Shailesh Tiwari and Tapan K. Mondal

10. ***Circa situm* Conservation of Tree Genetic Resources: A Case Study from the Central Western Ghats of Karnataka, India** **415**
Gudihalli M. Puneeth, Ravi Gowthami, Ramesh Vasudeva, Kerekoppa M. Laxmisha and Sunil Archak

11. **A Note on Taxonomy and Genetic Resource Potential of *Oryza meyeriana* var. *indandamanica*, a Rare Wild Relative of Rice from the Andaman Islands** **422**
K Pradheep, R Parimalan, K Joseph John, VA Muhammed Nissar, I Jaisankar, PP Thirumalaisamy and M Latha

12. **Effects of Different Treatments on Seed Germination and Breaking Seed Dormancy in Wild *Luffa* Species** **429**
Arvind Nagar, Amish K. Sureja, Chithra Pandey, Manjusha Verma, Lalit Arya3, Nakul Gupta and A.D. Munshi

13. **Effect of Nitrogen Nutrition Stress on Grain Weight, Starch Quality in Bread Wheat** **434**
Alka Bharati, Sachin Phogat, Jeet Roy, Amarjeet Kumar, Jayanthi Madhavan and Pranab K. Mandal

14. **Genetic Diversity Analysis of Bitter Gourd (*Momordica charantia* L.) Germplasm using Molecular Markers** **442**
Anupam Adarsh, Pankaj Kumar, Bishun D. Prasad, Randhir Kumar and Hemant K. Singh

15. **Comparative Analysis of Nutritional Values, Bioactive Compounds and Antinutrients in Tartary and Common Buckwheat Leaves** **449**
Nidhi Joshi, Kumkum Giri, Aditi Biswas, Pawanesh Tamta and Kuldip C. Verma

Plant Germplasm Registration Notice **455**
Anju Mahendru Singh and Anjali Kak

Guidelines to Authors

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.01

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Study on Genetic Variability and Correlation of Yield Traits in the F3 Population Derived from Wild Introgression Lines of Swarna/*Oryza nivara*

Kavitha Beerelli, Divya Balakrishnan*, Jyothi Badri, Senguttuvel P, Gireesh C, Ladhalakshmi D, Tuti MD, Padmavathi G, Subba Rao LV and Sarla Neelamraju

Abstract

Wild and related species are a source of wide genetic diversity. Back cross inbred line 166-2S derived from Swarna/*Oryza nivara* was crossed with Swarna to generate F_3 population. This F_3 population was raised in wet season of 2018 and data was collected for ten yield traits. Among all ten yield traits, single plant yield was highly associated with PH, TN, PTN, BM, TDM, HI, PL, PW and GW. HI was observed with a highly significant negative association with PH and BM. Positive skewness was observed for TN, PTN, SPY, BM, TDM and GW with platykurtic distribution and complementary gene action, suggesting an intense selection for better genetic gain. The panicle weight showed positive skewness with leptokurtic distribution, indicating the genetic regulation by only a few segregating genes. The negatively skewed and platykurtic distribution of PH, PL and HI indicated the governance of large number of dominant genes with duplicate interaction in the inheritance of these traits.

Keywords: Wild species, Rice, Back cross introgression, Variability, Correlation.

ICAR Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana, India.

***Author for correspondence:**

Divya.Balakrishnan@icar.gov.in

Received: 04/04/2022 **Revised:** 23/09/2023

Accepted: 25/09/2023

How to cite this article: Beerelli, K., Balakrishnan, D., Badri, J., Senguttuvel, P., Gireesh, C., Ladhalakshmi, D., Tuti, M.D., Padmavathi, G., Subba, R.L.V., Neelamraju, S. (2023). Study on Genetic Variability and Correlation of Yield Traits in the F3 Population Derived from Wild Introgression Lines of Swarna/*Oryza nivara*. Indian J. Plant Genetic Resources. 36(3), 341-345.
DOI: 10.61949/0976-1926.2023.v36i03.01

Introduction

Rice is a highly popular food grain, especially in Asian countries. Rice grain generally contains 75 to 80% starch, 12% water and 7% protein (Hossain *et al*., 2015), hence it is a main energy and food source for billions of people around the world. Global production of rice is about 800 million tonnes with 4,841 kg/ha productivity and grown in 117 countries covering a total area of 165.25 million hectares (FAOSTAT, 2021). Three-fold increase in global rice production was observed between 1961 and 2019, in the range of 215–755 million tonnes; with 80% of the production from Asian continent (Bin Rahman, and Zhang, 2023). Therefore the improvement in rice production is of utmost priority for food and nutritional security of Asian population.

Yield improvement is very challenging in rice due to multiple component traits involved with complex interaction effects. The correlation coefficient method is fundamental to understanding the associations among individual contributing traits in a population (Sravan *et al*., 2012). Detection of correlation coefficients between yield and its component characters are essential since rice grain yield is a complex trait with polygenic inheritance (Hossain *et al*., 2015, Kishore *et al*., 2015, Tiwari *et al*., 2019). As component traits influence the direct selection process for best lines, the trait association studies are helpful for the estimation of the correlation and the individual importance of each trait to improve the yield levels. The distribution properties of skewness

and kurtosis represent the insight about the nature of gene action and number of genes controlling each trait, respectively (Sheshaiah *et al*., 2018). As wild species are known for new genes for trait Improvement, we studied the variability and correlation of yield traits in wild introgression lines. The present study was carried out to evaluate the yield traits in a segregating population derived from Back cross introgression line (BIL) from a wild accession of *Oryza nivara*. The correlation among yield traits and frequency distribution patterns for yield and its component traits was estimated in this study.

Materials and Methods

The material for this study included F_3 generation with a population size of 180 lines, developed from a cross between 166-2S x Swarna (Figure 1). BIL 166-2S was derived from the wild introgression line 166S (Kavitha *et al*., 2019) developed from Swarna´ *O. nivara* (IRGC81848) cross (Swamy *et al*., 2011). BIL 166-2S was selected based on high grain number, filled grains and panicle weight. It was identified as a stable line with high yield and less than 10% of donor introgression (Divya *et al*., 2016). This BIL also showed highest water use efficiency with stable net photosynthesis rate in both seasons of *Kharif* 2013 (20.16 ± 0.9 µmol (CO^2) m^{-2} s^{-1}) and Rabi 2014 (19.42 ± 1.8 µmol (CO^2) m^{-2} s^{-1}) (Rao *et al*., 2018). 166-2S was crossed with recurrent parent Swarna and developed a BIL population. The F_3 population was grown in Augmented design at Indian Institute of Rice Research (IIRR) farm, Rajendranagar, Hyderabad, during wet season of 2018.

Phenotyping was carried out based on standard evaluation system of rice (SES), IRRI (2013) with two replications and the average of five plants in each replication was taken for yield traits like plant height (PH), tiller number (TN), productive tiller number (PTN), single plant yield (SPY), biomass (BM), panicle length (PL), panicle weight (PW), thousand-grain weight (TGW) and other derived traits like total dry matter (TDM) and harvest index (HI).

Data was analyzed and mean, range, skewness, kurtosis, frequency distribution and correlation were estimated with the software STAR v2.0.1. Correlation values were taken using Pearson's product-moment correlation method at significant levels of $^{*}p = 0.05\text{-}0.001$ and $^{**}p \geq 0.001$.

Results and Discussion

The minimum, maximum, median, range, variation, standard deviation, critical value, skewness and kurtosis values of 10 yield traits in F_3 population were tabulated in Table 1. The frequency distribution of yield traits were tabulated in Figure 2. The range of each trait was determined based on variability between minimum and maximum mean values of a trait. The maximum variability was observed for total dry matter with a range of 67.96 and minimum variability was observed for the trait panicle weight with a range of 7.22. The variance value for each trait was obtained by taking the average of the square deviation from mean value in the population. The variance value is the square of standard deviation was recorded and the highest variance was observed for plant height (194.67) and lowest variance for panicle weight (0.80). The CV of each trait in the population shows the percent of standard deviation to the total mean of a trait in the population, the lowest CV observed for panicle length (8.49) followed by plant height (11.99) and harvest index (12.90), the highest CV observed for biomass (40.01), single plant yield (38.04), total dry matter (35.67).

The trait genetics can be explored in detail using third and fourth-degree statistics i.e. skewness and kurtosis in segregating generations (Savitha and Ushakumari, 2015). The present study's traits exhibit positive and negative skewness values. Traits observed with positive skewness indicate that more proportion of individuals present in the lower end of distribution but transgressive segregants were also obtained for these traits. Hence, the selection of single plants from the transgressive segregants will improve the positively skewed traits (Priyanka *et al*., 2019). Highly positive skewness was observed with the traits TN, PTN, SPY, BM, TDM, PW and GW. Higher phenotypic values were observed in segregating populations for a number of tillers, productive tillers, single plant yield, biomass, total dry matter, panicle weight and grain weight compared to the parents. Selection through extreme genotypes in the frequency distribution improve the positively skewed traits. Similar results were observed for TN, PTN, PL and SPY by studies reported by Kiran *et al*., (2012); Rani *et al*., (2016). Whereas negatively skewed distribution observed for PH, PL and HI, as reported previously by Vijaya and Shailaja., 2016. In this case selection can be made from lower extreme of the distribution to get the dwarf plants for yield improvement. In the case of PL, many plants had high grain weight with high panicle length (Priyanka *et al*., 2019). Kurtosis values are less than three and showed platykurtic distribution for all traits except for the trait panicle weight which showed leptokurtic distribution (Raghavendra and Hittalmani , 2015) with high kurtosis value of 6.61.

These skewness and kurtosis values explain the gene action of a particular trait in the segregating populations (Jayaramachandran *et al*., 2010). The traits TN, PTN, SPY, BM, TDM and GW with positive skewness and platykurtic distribution explain that trait controlled by many genes with complementary gene action and needed extensive selection for vast genetic gain. The panicle weight showed positive skewness with leptokurtic distribution and was controlled by only a few segregating genes. The other traits PH, PL and HI showed negatively skewed and platykurtic distribution (Raghavendra and Hittalmani, 2015) governed by large number of dominant genes with duplicate gene interaction in inheritance (Sheshaiah *et al*., 2018).

Correlation was performed for the ten yield traits in F_3 population of 166-2S ´Swarna and correlation studies

Table 1: Descriptive statistics for the yield-related traits in F_3 population of 166-2S x Swarna

Trait	*Minimum*	*Maximum*	*Mean*	*Median*	*Range*	*Variance*	*Standard deviation*	*Critical value*	*Skewness*	*Kurtosis*
PH	81.33	149.00	116.37	116.50	67.67	194.67	13.95	11.99	-0.07	-0.31
TN	5.00	24.00	11.39	11.00	19.00	10.19	3.19	28.04	1.01	1.58
PTN	5.00	24.00	11.20	10.67	19.00	9.98	3.16	28.21	1.06	1.85
SPY	5.67	53.67	21.48	19.67	48.00	66.78	8.17	38.04	1.26	2.02
BM	3.90	41.73	17.11	16.27	37.83	46.87	6.85	40.01	0.83	0.79
TDM	10.77	78.73	38.60	36.10	67.96	189.54	13.77	35.67	0.85	0.38
HI	37.98	72.57	55.90	56.32	34.59	51.98	7.21	12.90	-0.03	0.02
PL	18.00	29.17	24.28	24.43	11.17	4.25	2.06	8.49	-0.34	0.39
PW	0.86	8.08	2.68	2.69	7.22	0.80	0.90	33.41	1.42	6.61
GW	8.70	39.90	21.00	19.70	31.20	49.73	7.05	33.58	0.62	-0.08

PH- plant height, TN- Tiller number, PTN- Productive tiller number, SPY- Single plant yield, BM- Biomass, TDM- Total dry matter, HI- Harvest index, PL- Panicle length, PW-Panicle weight, TGW-Thousand grain weight.

Table 2: Correlation for yield traits in F_3 population of 166-2S x Swarna

	PH	*TN*	*PTN*	*SPY*	*BM*	*TDM*	*HI*	*PL*	*PW*	*GW*
PH	1.00									
TN	0.08									
PTN	0.11*	0.98**								
SPY	0.24**	0.70**	0.70**							
BM	0.44**	0.62**	0.63**	0.68**						
TDM	0.36**	0.73**	0.73**	0.93**	0.90**					
HI	-0.30**	-0.01	-0.01	0.25**	-0.49**	-0.09				
PL	0.42**	0.10*	0.10*	0.31**	0.22**	0.30**	0.05			
PW	0.04	0.03	0.04	0.27**	0.13*	0.22**	0.14*	0.52**		
TGW	0.22**	0.00	0.00	0.18**	0.17**	0.19**	0.00	0.04	-0.09	1.00

PH- plant height, TN- Tiller number, PTN- Productive tiller number, SPY- Single plant yield, BM- Biomass, TDM- Total dry matter, HI- Harvest index, PL- Panicle length, PW-Panicle weight, TGW-Thousand grain weight.

Figure 1: Picture for plant of F1 along with parents, 166-2S and Swarna (left side). Seed picture of 166-2S and Swarna (right side)

provide an effective basis of phenotypic selection in plant populations (Chamundeswari *et al*., 2014). The simple correlation coefficient values among ten yield traits were presented in Table 2. Highly significant correlation was observed for single plant yield with the traits PH, TN, PTN, BM, TDM, HI, PL, PW and GW and it was reported previously by Ramanjaneyulu *et al*., (2014). The results reported by Archana *et al*., (2018) showed a positive significant association of SPY with PTN, PL, PW, TGW and HI. The traits TN, PTN and TGW also showed positive association with SPY

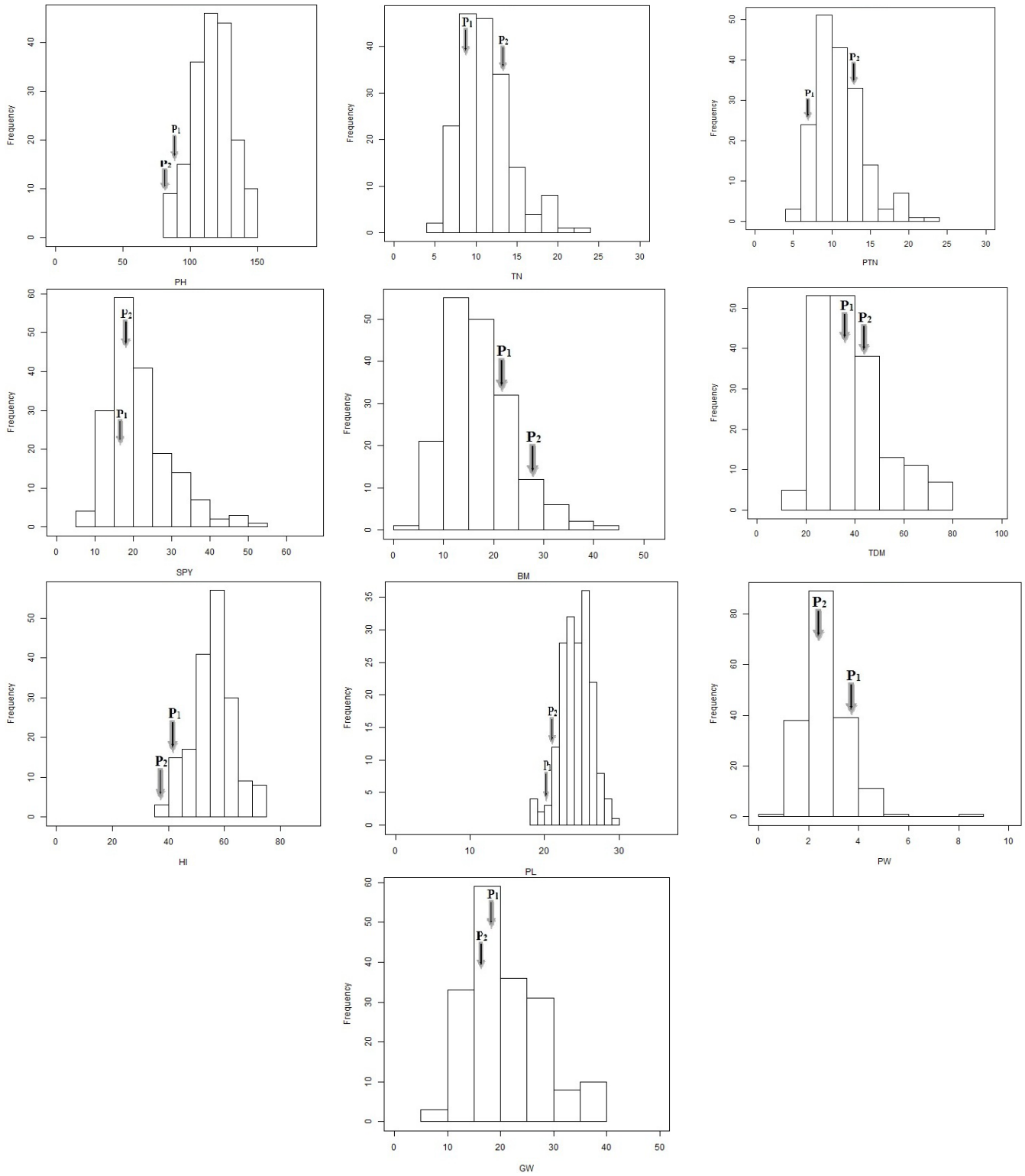


Figure 2: Frequency distribution of F3 population of 166-2S x Swarna for ten yield traits pH plant height, TN- Tiller number, PTN- Productive tiller number, SPY- Single plant yield, BM- Biomass, TDM- Total dry matter, HI- Harvest index, PL- Panicle length, PW-Panicle weight, GW- grain weight.

(Tiwari *et al*., 2019; Sreedhar and Reddy, 2019) it was observed that there is a highly significant negative association of HI with PH and BM in this population and it was previously reported by Dhavaleshvar *et al*., (2019).

20 lines C5-11, C5-172, C5-192, C5-224, C5-152, C5-174, C5-238, C5-133, C5-80, C5-102, C5-240, C5-3, C5-28, C5-232, C5-119, C5-207, C5-93, C5-43 and C5-113 showed more than 15% of improvement in grain yield over both the parents

by significant pairwise mean comparison of yield. Three genotypes C5-24, C5-172 and C5-43 with multiple yield traits contribute for yield and found promising for further breeding programmes.

Acknowledgments

This research was conducted in project (ABR/CI/BR/28) Exploring wild introgression lines and mutants to identify novel genes/QTLs for yield contributing traits and ICAR-National Professor Project (F.No: Edn/27/4/NP/2012-HRD) funded by Indian Council of Agricultural Research, New Delhi, India. The authors are grateful to the Director, ICAR-IIRR for providing facilities.

References

Archana RS, Sudha Rani M, Vishnu vardhan KM and G Fareeda, (2018) Correlation and path coefficient analysis for grain yield, yield components and nutritional traits in rice (*Oryza sativa* L.). *Int.J. of Chem. Studies* **6(4)**: 189-195.

Bin Rahman ANM and J Zhang (2023). Trends in rice research: 2030 and beyond. Food and Energy Sec. 12, e390. *https://doi.org/10.1002/fes3.39*

Kavith, B, Divya B, Malathi S, Krishnamraju A, Venkateshwara Rao Y and N Sarla (2019) Evaluation of yield and seedling vigour related traits of Swarna/*Oryza nivara* backcross introgression lines under three environment conditions. *Eco. Gen. and Genom.*, 11, 100036.

Chamundeswari N, Satyanarayana PV, Manasam Y, Uma sundari P, Ravi kumar B, Girija Rani M, Ramana Rao P V and K Vishnuvardhan (2014) Correlation and path analysis for yield and quality traits in direct seeded Rice (*Oryza sativa* L.). *Life sci. int. res.* **1(1)**:331 – 337.

Dhavaleshvar M, Malleshappa C and BM Dushyanth Kumar (2019) Variability, correlation and path analysis studies of yield and yield attributing traits in advanced breeding lines of rice (*Oryza sativa* L.)*, Int. j. of pure and app. Biosci.* **7(1)**: 267-273.

Divya B, Subrahmanyam D, Badri J, Raju AK, Rao VY, Kavitha B, Sukumar M, Malathi S, Revathi P, Padmavathi G, Babu VR, N Sarla (2016) Genotype× environment interactions of yield traits in backcross introgression lines derived from *Oryza sativa* cv. Swarna/ *Oryza nivara*. *Fron..s in plant Sci.* **7(1530)**: 10-3389.

FAOSTAT (2022). Statistical data base. Food and Agriculture Organizations of the United Nations. Rome, Italia.

Hossain S, Haque M and J Rahman (2015) Genetic variability, correlation and path coefcient analysis of morphological traits in some extinct local Aman rice (*Oryza sativa* L). *Rice Res*: **3**:158. *doi:10.4172/2375-4338.1000158*

Jayaramachandran M, Kumaravadivel N, Eapen S and G Kandasamy (2010) Gene Action for yield attributing characters in segregating generation (M2) of Sorghum (Sorghum bicolor L.). *Elec. J. of plant breed.* **1(4)**. pp. 802-805.

Kiran KK (2012) Genetic variability for grain yield, its components and inheritance of resistance to BPH in two F_2 populations of rice (*Oryza sativa* L.). M. Sc. (Agri.) Thesis, Univ. Agril. Sci., Bangalore.

Kishore N, Srinivas T, Nagabhushanam U, Pallavi M and S Sameera (2015) Genetic variability, correlation and path analysis for yield and yield components in promising rice (*Oryza sativa* L.) genotypes. *SAARC J. of Agric.* **13(1)**: 99-108.

OECD-FAO (2021). OECD-FAO Agricultural Outlook 2021-2030. OECD Publishing. *https://doi.org/10.1787/19428846-en*

Priyanka AR, Gnanamalar RP, Banumathy S, Senthil N and G Hemalatha (2019) Genetic variability and frequency distribution studies in F_2 segregating generation of rice. *Elec. J. of plant breed.* **10(3)**: 988-994.

Raghavendra P and S Hittalmani (2015) Genetic parameters of two BC_2F_1 populations for development of superior male sterile lines pertaining to morpho-floral traits for aerobic rice (*Oryza sativa* L.). *SAARC J. of Agric.* **13(2)**: 198-213.

Ramanjaneyulu AV, Gouri Shankar V, Neelima TL and D Shashibhusahn (2014) Genetic analysis of rice (*Oryza sativa* L.) genotypes under aerobic conditions on alfisols. *SAARC J. of Agric.* **46(1)**: 99-111.

Rani CS, Anandakumar CR, Raveendran M, Subramanian KS and S Robin (2016) Genetic variability studies and multivariate analysis in F_2 segregating populations involving medicinal rice (*Oryza sativa* L.) cultivar Kavuni. *Int. J.*of *Agric.Sci.* **8(15)**: 1733-1735.

Rao YV, Balakrishnan D, Addanki KR, Mesapogu S, Kiran TV, Subrahmanyam D, Sarla N and SR Voleti (2018) Characterization of backcross introgression lines derived from *Oryza nivara* accessions for photosynthesis and yield. *Phy. and Mol. Biol. of Plants* **24(6)**: 1147-1164.

Savitha P and R Usha (2015) Genetic variability studies in F_2 and F_3 Segregating generations for yield and its components in rice (*Oryza sativa* L.). *Indian J. of Sci. and Technol.* **8(17)**: 1-7

Sheshaiah BM, Dushyantha Kumar S, Gangaprasad TH, Gowda GN, Hosagoudar and HE Shashidhar (2018) Studies on variability and frequency distribution of yield and yield related traits in F_2 population of rice (*Oryza sativa* L.). *Int.J. of Curr. Microbiol. and Appl.*Sc. **7(09)**: *doi: https://doi.org/10.20546/ijcmas.2018.709.*

Sravan T, Rangare N, Suresh B and S Kumar (2012) Genetic variability and character association in rainfed upland rice (*Oryza sativa* L.), *J. of Rice Res.* 5, pp. 24–29.

Sreedhar S and R Uma Reddy (2019) Association studies for yield and its traits in rice (*Oryza sativa* L.) genotypes. *Int.J. of Curr. Microbiol. and Appl.*Sc. **8(01)**: 2337-2342.

Standard evaluation system (SES) for rice. (2013) fifth ed. IRRI, Philippines.

Tiwari DN, Tripathi SR, Tripathi MP, Khatri N and BR Bastola (2019) Genetic variability and correlation coefficients of major traits in early maturing rice under rainfed lowland environments of Nepal. *Adv. in Agric.e*. 2019: 1-9

Vijaya I and H Shailaja (2016) Assessment of genetic parameters for yield and its related traits in F2 population of KRH-4 hybrid rice (*Oryza sativa* L.). *Int.l J. of Agric Sci.e and Res.* **6(3)**: 87 – 96.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.02

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Characterization and Evaluation of Faba Bean (*Vicia faba* L.) Germplasm Collected from Kashmir, India

Sheikh M. Sultan[1*], Susheel K. Raina[2], Suheel Ahmad[3] and Sheeraz S. Bhat[4]

Abstract

Faba bean (*Vicia faba*) is an ancient legume crop with high protein content of around 27%. In this study 39 local faba bean genotypes differing in seed shape, size and color were collected from different areas of Kashmir valley and evaluated during three successive *rabi* growing seasons of 2019–20, 2020–21 and 2021–22. Data were recorded on ten agro-morphological traits *viz*., plant height, number of branches/plant, days to 50% flowering, days to 80% maturity, pod length, pod width, number of seeds/pod, pod yield/plant, seed yield/plant and 100-seed weight. Average plant height ranged from 55.8 to 91.3 cm, pod length 5.2 to 9.7 cm, pod width 1.1 to 1.8 cm, seed yield/plant 18.748 to 42.050 g and 100-seed weight 41.118 to 89.966 g. Pod yield/plant evaluated during one year of 2021–22 only ranged from 43.633 to 199.930 g with a mean value of 99.981 g. The results have indicated significant variability in most of these traits. Flattened, medium-sized, beige seeds were more common traits in these faba bean genotypes. Lower mean plant height, number of branches/plant, pod length and seed yield/plant were recorded in 2021–22 than the other two experimental years due to lower rainfall and higher mean temperatures during the active vegetative, flowering and pod filling stages. Principal component and cluster analysis revealed three distinct clusters mainly on the basis of greater agro-morphological similarity. Interestingly, 8 genotypes with the highest individual 100-seed weight were clustered in a separate group with an average 100-seed weight of 83.997 g. Besides, this cluster recorded highest average plant height, pod length, pod width and seed yield/plant as well; all these traits showed significant positive correlations with each other. Therefore, these traits should be considered when selecting faba bean genotypes to improve the region's crop performance.

Keywords: Agro-morphological traits, Cluster analysis, Genetic diversity, Principal components, *Vicia faba*.

[1,2]ICAR-NBPGR, Regional Station, Srinagar, Jammu & Kashmir, India

[3,4]ICAR-IGFRI, Regional Station, Srinagar, Jammu & Kashmir, India

***Author for correspondence:**

mohmmad.sheikh@icar.gov.in

Received: 17/08/2022 **Revised:** 13/07/2023

Accepted: 17/10/2023

How to cite this article: Sultan, S.M., Raina, S.K., Ahmad, S., Bhat, S.S. (2023). Characterization and Evaluation of *Faba* Bean (*Vicia faba* L.) Germplasm Collected from Kashmir, India. Indian J. Plant Genetic Resources. 36(3), 346-355. **DOI:** 10.61949/0976-1926.2023.v36i03.02

Introduction

Faba bean (*Vicia faba* L.) also fava bean, broad bean, field bean or horse bean is a multipurpose ancient legume crop belonging to the family *Faba*ceae have a high protein content and biomass and is used as human food and animal feed (Musallam *et al.*, 2004). Mature seeds of faba bean are rich in proteins (26.1%), carbohydrates (58.3%), and dietary fiber (25.0%) (USDA, 2021), the protein content ranging from 22.7 to 34.7% (Martineau-Côté *et al.*, 2022). Faba beans also contain a variety of bioactive compounds such as phenolics and flavonoids with antioxidant activity (Valente *et al.*, 2018). However, faba beans also contain anti-nutritional factors such as lectins, saponins, trypsin inhibitors, phytic acids, condensed tannins, etc., that are believed to undermine its biological value (Revilla, 2015). Consuming faba beans may cause a condition known as 'favism', which is a severe form of hemolytic anemia (Mínguez & Rubiales, 2021), the highest incidence of this disease has been reported in the Mediterranean region. Faba beans, a fresh vegetable, represent a traditional food in several Mediterranean countries and are consumed in large quantities in countries such as China, Turkey, Egypt, Ethiopia, and Central America. In fact, it is a common breakfast food in the Middle East, Mediterranean

region, China, and Ethiopia (Dhull *et al.*, 2021). Faba beans also play an important role in crop rotations, effective nitrogen fixation and soil improvement (Ye *et al.*, 2003) and are considered among the most efficient legumes in terms of nitrogen fixation (Phillips, 1980). Due to its very efficient nitrogen fixation capability, it is used as a green manure globally (Duc *et al.*, 2015), providing about 90% of the plant's nitrogen needs (Hauggaard-Nielsen *et al.*, 2009), probably the highest value among grain legumes. About half of the crop nitrogen content is left in the field for the next crop (Watson *et al.*, 2017).

Faba bean is believed to have been domesticated some 10,000 years BC in the near east, where archeological findings of domestic (Cubero, 1974) and wild specimens were discovered (Caracuta *et al.*, 2016). Ancient faba bean remains have been found in northwest Syria and Turkey (Tanno and Willcox, 2006). Faba bean cultivation spread to Anatolia and then Europe via the Mediterranean coast and to other countries, including India and China via Mesopotamia (Cubero, 2011). Today, the faba bean is considered the fourth most important cool season food legume after chickpeas, lentils, and peas as is grown on 2.57 million ha area with a total production of 5.4 million tons in 2019 (FAOSTAT, 2021) and cultivated worldwide (Mínguez & Rubiales, 2021). China is the leading producer of *faba* beans, followed by Ethiopia; these two countries represent about 50% of the total global production. Mediterranean countries, Ethiopia, Egypt, Afganistan, India, Northern Europe and Northern Africa are other major producers of *faba* bean (Rahate *et al.*, 2020). *Faba* bean gained specific adaptations to different environments and uses during its dispersal. These adaptations are reflected in plant architecture and seed size, weight, and shape (Alghamdi *et al.*, 2012). Accordingly, *faba* bean is separated into four groups based on seed size: major (large flattened or broad bean with 100–200 g/100 seed weight), equine (horse bean with 60–100 g/100 seed weight), minor (tic bean with ellipsoidal seed having 40–60 g/100 seed weight), and *paucijuga* (regarded as primitive one with 31–40 g/100 seed weight) (Cubero 1974, Serradilla *et al.*, 1993). The largest seeded type (major) is generally found in south Mediterranean countries and China. The medium-sized type (equina) emerged in the Middle East, North Africa, and Australia, whereas small-seeded types (minor and *paucijuga*) are prevalent in Ethiopia and northern Europe (Duc, 1997). Some workers have classified *paucijuga* as a sub-species. *V. faba* var. *paucijuga* has dehiscent pods as do the other groups but to a much lesser extent, it is self-fertile with short stature and is closest to wild type in traits (Ladizinsky, 1998), occurring in the Afghan-north Indian subcontinent region. Short stem, small leaves and seeds (100 seed weight less than 25 g) are primitive characteristics. The small seed/pod features dominate over the larger, more recent seed types (Cubero and Nadal, 2005). Owing to its numerous uses, great nutritional quality and ability to grow over a broad range of climatic and soil conditions, faba bean is appropriate for sustainable agriculture in many marginal areas (Nadal *et al.*, 2003), and the crop has gained greater global attention in recent years. Its production is poised for rapid growth because of demand for plant-based protein products.

Faba bean is a minor crop in India, grown in the states of Bihar, Uttar Pradesh, Rajasthan, Madhya Pradesh, West Bengal, Assam, Manipur and Nagaland (Singh *et al.* 2012) mainly as a *rabi* crop in plain areas and during rainy *kharif* season in hilly and mountainous regions (Singh and Bhatt, 2012). In Kashmir, it is cultivated in several areas on a limited scale, mainly as *rabi* crop for household consumption as a fresh vegetable and occasionally as a pulse. Locally known as 'Baghla', which is similar to 'Bakla', the Turkish and Persian name of the crop, faba bean is considered as a rustic crop able to withstand environmental and ecological vagaries. It is surprising, however, that not much work has been done on local faba bean genetic resources and their cultivation in Kashmir as there is scanty information available in literature in this regard. The present study was therefore undertaken to evaluate the field performance of 39 faba bean genotypes we collected from different areas of Kashmir by using nine agro-morphological traits. The study of genetic variation in morphological and agronomic traits among these local faba bean genotypes is imperative for their efficient utilization and for their effective conservation.

Materials and Methods

Plant materials and field conditions

Thirty nine (39) genotypes of faba bean (*V. faba*) collected by us during the year 2019 from farmers in different areas of Kashmir, especially Budgam, Shopian and Pulwama districts at an altitudinal range of 1591–1881 masl (Table 1) were studied for their field performance. The experiments were conducted over three successive *rabi* growing seasons of 2019–20, 2020–21 and 2021–22 under rainfed conditions at the experimental farm of ICAR-NBPGR Regional Station Srinagar, Jammu and Kashmir (33°59′ N latitude, 74°47′ E longitude, 1639 m above sea level). The experimental site is on dry Karewa land with a pH of 6.23 and an average normal monthly rainfall of 52 mm (Table 2). These genotypes were sown during the third week of November each year with two check varieties HFB-1 and Vikrant in a randomized block design with three replications. In each replication, the genotypes were sown in two rows of 2 m length spaced at 45 cm and the individual plants in a row spaced at 15 cm. Recommended agronomic practices and protective measures were followed to grow a healthy crop. The experiments were supplied with granulated NPKS in the 40:20:20:20 kg/ha ratio at the time of land preparation. Weeding was done by hand whenever necessary.

Table 1: Geographical coordinates of collection sites of 39 faba bean (*Vicia faba*) genotypes collected from different areas of Kashmir, India (those with IC numbers have been conserved in National Gene Bank, ICAR-NBPGR New Delhi)

S No.	*Collection No./ Accession No.*	*Place of collection*	*Latitude*	*Longitude*	*Altitude (m)*
1	SHEIKH/SR-899 *IC-0637960*	Buchroo, Budgam	33° 58′	74° 49′	1610
2	SHEIKH/SR-900 *IC-0637961*	Buchroo, Budgam	33° 58′	74° 49′	1610
3	SHEIKH/SR-901 *IC-0637962*	Manchuwa, Budgam	34° 00′	74° 48′	1600
4	SHEIKH/SR-902 *IC-0637963*	Dangerpora, Budgam	34° 07′	74° 46′	1600
5	SHEIKH/SR-903 *IC-0637964*	Bugam, Budgam	33° 45′	74° 47′	1642
6	SHEIKH/SR-903A	Bugam, Budgam	33° 45′	74° 47′	1642
7	SHEIKH/SR-903B	Bugam, Budgam	33° 45′	74° 47′	1642
8	SHEIKH/SR-903C	Bugam, Budgam	33° 45′	74° 47′	1642
9	SHEIKH/SR-904	Bugam, Budgam	33° 45′	74° 47′	1642
10	SHEIKH/SR-905 *IC-0637965*	Hayatpora, Budgam	33° 58′	74° 44′	1630
11	SHEIKH/SR-906 *IC-0637966*	Nowbugh, Budgam	33° 56′	74° 48′	1634
12	SHEIKH/SR-906A	Nowbugh, Budgam	33° 56′	74° 48′	1634
13	SHEIKH/SR-907 *IC-0637967*	Nowpora, Budgam	34° 09′	74° 54′	1620
14	SHEIKH/SR-908 *IC-0637968*	Pehroo, Budgam	34° 00′	74° 46′	1591
15	SHEIKH/SR-909 *IC-0637969*	Wadipora, Budgam	33° 57′	74° 47′	1637
16	SHEIKH/SR-910 *IC-0637970*	Gowharpora, Budgam	33° 59′	74° 47′	1650
17	SHEIKH/SR-910A	Gowharpora, Budgam	33° 59′	74° 47′	1650
18	SHEIKH/SR-911	Nohmu, Pulwama	34° 02′	74° 48′	1620
19	SHEIKH/SR/SA/SS-912 *IC-0637971*	Trenz, Shopian	33° 46′	74° 55′	1860
20	SHEIKH/SR/SA/SS-913 *IC-0637972*	Haripora, Shopian	33° 45′	74° 54′	1800
21	SHEIKH/SR/SA/SS-913A	Haripora, Shopian	33° 45′	74° 54′	1800
22	SHEIKH/SR/SA/SS-914 *IC-0637973*	Aawneera, Shopian	33° 48′	74° 58′	1867
23	SHEIKH/SR-915 *IC-0637974*	Newa, Pulwama	33° 54′	74° 53′	1623
24	SHEIKH/SR-915A	Newa, Pulwama	33° 54′	74° 53′	1623
25	SHEIKH/SR/SA/SS-916 *IC-0637975*	Chitragam, Shopian	33° 46′	74° 58′	1696
26	SHEIKH/SR/SA/SS-917	Chitragam, Shopian	33° 46′	74° 58′	1696
27	SHEIKH/SR/SA/SS-918 *IC-0637976*	Zanipora, Shopian	33° 54′	74° 48′	1770
28	SHEIKH/SR/SA/SS-918A	Zanipora, Shopian	33° 54′	74° 48′	1770
29	SHEIKH/SR-919 *IC-0637977*	Kakapora, Pulwama	33° 56′	74° 55′	1608
30	SHEIKH/SR-920 *IC-0637978*	Gangoo, Pulwama	33° 52′	74° 53′	1655

31	SHEIKH/SR/SA/SS-921 *IC-0637979*	Kundalan, Shopian	33° 46′	74° 55′	1881
32	SHEIKH/SR/SA/SS-922	Naidgam, Shopian	33° 47′	74° 57′	1878
33	SHEIKH/SR/SA/SS-922A	Naidgam, Shopian	33° 47′	74° 57′	1878
34	SHEIKH/SR/SA/SS-922B	Naidgam, Shopian	33° 47′	74° 57′	1878
35	SHEIKH/SR/SA/SS-923 *IC-0637980*	Naidgam, Shopian	33° 47′	74° 57′	1878
36	SHEIKH/SR/SA/SS-923A	Naidgam, Shopian	33° 47′	74° 57′	1878
37	SHEIKH/SR-924	Mattan, Anantnag	33° 45′	75° 12′	1686
38	SHEIKH/SR-925	Mattan, Anantnag	33° 45′	75° 12′	1686
39	SHEIKH/SR-926	Mattan, Anantnag	33° 45′	75° 12′	1686

Table 2: Average monthly minimum and maximum temperatures and total rainfall during three experimental years of 2019-20, 2020-2021 and 2021-2022

Parameter	*Year*	*Nov.*	*Dec.*	*Jan.*	*Feb.*	*Mar.*	*Apr.*	*May*	*June*	*July*
Maximum temperature (°C)	2019-20	15.0	8.6	5.9	12.5	15.1	21.3	25.2	29.3	31.8
	2020-21	14.6	9.2	5.7	12.9	15.3	19.0	24.5	29.4	30.0
	2021-22	14.9	10.5	7.3	10.8	20.8	24.6	26.2	27.7	25.2
Minimum temperature (°C)	2019-20	1.9	-2.0	-2.0	-0.3	4.7	7.5	10.3	14.4	17.4
	2020-21	1.4	-2.0	-4.6	0.9	5.5	7.3	11.7	15.6	19.5
	2021-22	0.6	-2.0	-6.0	4.0	6.7	9.9	13.1	14.9	20.6
Total rainfall (mm)	2019-20	39	55	48	70	121	82	68	47	07
	2020-21	31	57	103.7	25.1	110.2	126.7	47.5	19.6	52.6
	2021-22	3.0	17.5	105	79	32	32.8	27.2	108	122

Recorded traits

At maturity, five guarded plants in each replication were used to measure the agro-morphological traits *viz*., plant height (cm), number of branches/plant, days to 50% flowering, days to 80% maturity, pod length (cm), pod width (cm), number of seeds/pod, pod yield/plant (g) and seed yield/plant (g). 100-seed weight (g) was also recorded in triplicate. Pod yield/plant was evaluated during the final year of the study *i.e*., 2021–22 only. To determine pod yield/plant, two harvests were carried out simultaneously for all the genotypes during the second fortnight of May 2022.

Statistical Analysis

Basic statistics of different quantitative traits was worked out on pooled data of all the traits studied excepting pod yield/plant, which was evaluated only during one year of 2021–22. Least significance difference (LSD) was worked out at p = 0.05 level of probability. Pearson's correlation determined correlations between different traits. The data were then analyzed by cluster and principal component analysis. Principal components analysis (PCA) was carried out on the correlation matrix, calculating the mean data of the genotypes. The genotypes in each resultant cluster were also analyzed for basic statistics. Statistical tests were carried out by the Statistical Package for Social Sciences (version 21) and OpenStat software.

Results and Discussion

A total of 39 *Vicia faba* bean genotypes (*V. faba*) were collected by us during an exploration and germplasm collection programme in 2019 from the farmers in different areas of Kashmir (Table 1). Most of these have been conserved in National Gene Bank at ICAR-NBPGR New Delhi. In Kashmir, *faba* bean is cultivated as a minor *rabi* crop mostly for household consumption. It is used as a vegetable either in the form of green pods or immature seeds, sometimes, its dried seeds are used as a pulse. Generally, these farmers maintain a mixture of seeds of different size, shape and color, most commonly being medium-sized beans of beige color. Seed shape varies from more or less rounded, angular to flattened, the latter being observed as dominant.

The average combined values of 9 out of 10 agro-morphological traits for the 39 *faba* bean genotypes evaluated during three successive years of 2019–20, 2020–21 and 2021–22 are given in Table 3. Pod yield/plant was only evaluated during the single year of 2021–22. The results revealed a wide range of differences, as evidenced by the characteristic ranges that especially include plant height (55.8–91.3 cm), number of branches per plant (1.2–3.3), pod length (5.2–9.7 cm), pod yield/plant (43.633–199.930 g), seed yield per plant (18.700–42.000 g) and 100-seed weight

Table 3: Mean performance of 39 local *faba* bean (*Vicia faba*) genotypes evaluated under rainfed conditions of Kashmir, India combined over three *rabi* seasons of 2019-20, 2020-2021 and 2021-2022

S No.	Genotype	PH (cm)	DTF	DTM	NB	PL (cm)	PW (cm)	SP	SY (g)	SW (g)	*PY (g)
1	SHEIKH/SR-899	61.6	142.5	195.2	1.5	5.8	1.5	2.4	32.246	51.936	89.217
2	SHEIKH/SR-900	72.3	141.5	198.0	2.1	6.9	1.5	3.2	33.776	60.103	49.217
3	SHEIKH/SR-901	74.2	142.7	194.7	2.2	7.9	1.8	3.2	38.696	88.684	85.017
4	SHEIKH/SR-902	74.5	139.5	198.5	2.5	8.5	1.5	3.1	36.835	84.497	80.500
5	SHEIKH/SR-903	73.9	141.5	196.5	2.0	8.3	1.6	2.9	25.716	70.091	95.933
6	SHEIKH/SR-903A	57.3	142.0	194.0	1.6	7.0	1.1	2.9	27.639	60.350	161.730
7	SHEIKH/SR-903B	56.1	140.5	194.0	2.0	5.7	1.2	2.8	33.400	67.318	119.950
8	SHEIKH/SR-903C	55.8	141.2	196.5	1.5	5.2	1.1	2.7	18.748	54.950	82.650
9	SHEIKH/SR-904	68.4	141.5	196.5	2.5	7.8	1.5	2.9	26.301	69.730	153.630
10	SHEIKH/SR-905	66.9	142.7	196.0	2.0	7.2	1.5	2.7	36.553	71.282	198.930
11	SHEIKH/SR-906	73.6	143.5	194.2	3.1	7.3	1.4	2.9	32.559	51.048	97.433
12	SHEIKH/SR-906A	65.4	145.0	197.2	2.7	5.9	1.3	3.4	29.224	50.082	199.930
13	SHEIKH/SR-907	67.9	143.2	194.0	2.6	7.6	1.5	3.1	30.404	64.958	108.630
14	SHEIKH/SR-908	71.6	141.5	193.0	2.3	7.5	1.4	2.7	29.952	59.665	58.100
15	SHEIKH/SR-909	75.8	144.5	195.7	2.0	6.7	1.4	2.8	25.669	66.846	67.400
16	SHEIKH/SR-910	72.7	144.5	195.2	3.0	6.6	1.4	3.1	32.874	51.832	108.180
17	SHEIKH/SR-910A	75.0	142.0	199.0	2.8	6.8	1.2	2.8	25.000	45.90	99.683
18	SHEIKH/SR-911	78.2	145.5	196.0	2.7	5.9	1.2	3.1	35.238	48.661	97.200
19	SHEIKH/SR/SA/SS-912	91.3	144.0	196.0	3.3	9.2	1.7	2.8	39.604	67.784	121.700
20	SHEIKH/SR/SA/SS-913	83.1	142.0	200.0	2.8	9.7	1.8	3.1	36.500	81.333	82.000
21	SHEIKH/SR/SA/SS-913A	65.7	142.0	197.5	2.3	7.7	1.7	3.2	34.552	52.850	85.300
22	SHEIKH/SR/SA/SS-914	84.7	142.0	199.0	2.4	8.5	1.6	2.5	40.254	72.248	80.133
23	SHEIKH/SR-915	74.2	144.0	195.5	1.9	7.9	1.6	3.0	42.050	82.587	109.130
24	SHEIKH/SR-915A	72.8	144.0	197.2	2.4	7.2	1.2	3.1	19.137	56.900	91.850
25	SHEIKH/SR/SA/SS-916	72.7	143.2	197.7	2.7	6.9	1.4	3.1	33.063	45.343	78.350
26	SHEIKH/SR/SA/SS-917	69.9	142.5	198.5	1.2	8.2	1.7	3.3	37.386	79.563	80.800
27	SHEIKH/SR/SA/SS-918	70.8	143.0	199.0	2.6	7.3	1.8	2.7	34.859	70.408	110.250
28	SHEIKH/SR/SA/SS-918A	82.7	141.0	199.0	2.4	8.9	1.5	2.2	35.220	79.200	90.350
29	SHEIKH/SR-919	70.4	146.0	196.5	2.5	7.3	1.4	3.1	30.940	49.535	59.600
30	SHEIKH/SR-920	76.9	141.5	197.5	2.2	8.9	1.7	2.8	41.725	89.966	89.433
31	SHEIKH/SR/SA/SS-921	74.9	143.2	196.2	2.4	7.9	1.5	3.2	28.915	65.182	91.000
32	SHEIKH/SR/SA/SS-922	73.5	143.5	196.2	3.3	6.5	1.4	3.1	27.997	52.149	87.233
33	SHEIKH/SR/SA/SS-922A	60.6	145.5	199.0	2.1	5.2	1.1	2.7	27.600	42.950	105.870
34	SHEIKH/SR/SA/SS-922B	62.2	143.0	196.0	2.3	5.8	1.1	3.2	22.876	41.118	43.633
35	SHEIKH/SR/SA/SS-923	68.1	147.5	194.5	2.7	6.8	1.4	3.2	26.787	49.505	107.370
36	SHEIKH/SR/SA/SS-923A	69.7	143.5	197.5	3.1	6.2	1.3	3.4	24.988	62.844	119.430
37	SHEIKH/SR-924	63.2	144.5	195.5	2.2	8.1	1.8	2.9	21.278	86.146	102.470
38	SHEIKH/SR-925	65.1	142.5	198.0	2.8	6.4	1.4	2.9	41.724	42.401	106.470
39	SHEIKH/SR-926	69.1	142.5	196.5	2.3	6.1	1.1	3.4	26.220	41.866	103.570
Mean		70.8	142.5	196.0	2.4	7.2	1.4	2.9	31.397	62.302	99.981
Minimum		55.8	139.0	193.0	1.2	5.2	1.1	2.2	18.748	41.118	43.633
Maximum		91.3	147.0	200.0	3.3	9.7	1.8	3.4	42.050	89.966	199.930
CV (%)		10.7	1.1	0.9	20.2	15.4	14.9	9.2	19.800	23.0	33.1
LSD at 0.05		8.6	4.3	4.3	1.3	1.9	0.4	0.6	11.885	27.605	75.4
Mean values for check varieties											
HFB-1		55.4	141.8	196.5	2.3	5.1	1.1	3.0	17.585	26.526	69.700
Vikrant		52.5	142.1	196.8	1.8	4.3	1.2	2.7	20.018	26.713	68.133

PH = Plant height, DTF = Days to 50% flowering, DTM = Days to 80% maturity, NB = Number of branches/plant, PL = Pod length, PW = Pod width, SP = Seeds/pod, SY = Seed yield/plant, SW = 100 - Seed weight and PY = Pod yield/plant

*Single year data of 2021- 2022

(41.100–89.966 g). Significant differences have been recorded among various genotypes in these traits. The results suggest the presence of a wide range of genetic variability among these genotypes and highlight the genetic refinement that could be possible with the use of such a genetic pool for breeding. This conclusion agrees with those of Elshafei *et al.* (2019) and Ton *et al.* (2021), who reported a significant variance in various agronomic characteristics, especially of plant height, number of branches per plant, seed yield per plant and 100-seed weight in *Vicia faba* beans in their respective studies. The seed coat color of greenish, brown, purple, beige, chocolate or black was observed in freshly harvested plants. The seeds also varied in shape and size (Figure 1). Flattened, medium-sized, beige seeds were more common traits in these *Vicia faba* bean genotypes. As indicated in Table 3, the 100-seed weight ranged widely from 41.118–89.966 g (CV 23%), implying sufficient variability in this trait.

All the 39 faba bean genotypes studied exhibited indeterminate or semi-determinate growth habit. The apical vegetative growth continued even after the plants entered the reproductive phase and flowering ensued and flowers appeared on short or long peduncles laterally (Figure 1).

Figure 1: Representative faba bean (*V. faba*) inflorescences of 39 local genotypes with flowers showing anthocyanin coloration and striations. Note also variability in seed shape, size and color in these genotypes

The terminal vegetative growth stopped as soil moisture became limiting. The flower color of white with black spot on wing petal was observed in all the genotypes studied. In some genotypes, standard petal showed some purplish or reddish anthocyanin coloration. Streaks of medium color intensity were observed on standard petal in nearly all the genotypes studied. However, white flowers known to be a pleiotropic effect of an allele for very low tannins in the seed testa were not observed in any of these 39 genotypes.

A perusal of the data recorded in Table 4 suggests that the mean plant height, number of branches/plant, pod length and seed yield/plant in the third year of evaluation *viz*., 2021-22 were lower as compared to other two years of 2019–20 and 2020–21. This may be due to lower rainfall and higher mean temperatures during the active vegetative,

Table 4: The means and range of variation of 39 local faba bean (*Vicia faba*) genotypes evaluated under rainfed conditions of Kashmir, India during three years of 2019-20, 2020-2021 and 2021-2022

Trait	*Year*	*Mean*	*Range*	*Best check value*
Plant height (cm)	2019-20	82.8	61.8–92.2	67.4 (HFB-1)
	2020-21	73.7	58.5–92.2	54.1 (HFB-1)
	2021-22	57.0	45.0–69.0	44.7 (HFB-1)
Days to 50% flowering	2019-20	139.5	139–142	140 (Vikrant)
	2020-21	142.2	137–145	140.7 (HFB-1)
	2021-22	144	140–151	142 (Vikrant)
Days to 80% maturity	2019-20	198.5	195–199	193 (HFB-1)
	2020-21	198.2	196–200	197 (HFB-1)
	2021-22	195	188–199	196 (HFB-1)
No. of branches/ plant	2019-20	2.2	1.1–3.6	2.7 (HFB-1)
	2020-21	2.6	1.0–4.3	2.2 (HFB-1)
	2021-22	2.0	1.0–3.1	2.5 (HFB-1)
Pod length (cm)	2019-20	8.1	5.7–10.5	4.6 (HFB-1)
	2020-21	7.9	5.7–10.8	6.1 (Vikrant)
	2021-22	5.9	3.7–8.8	5.4 (HFB-1)
Pod width (cm)	2019-20	1.7	1.4–2.1	1.4 (Vikrant)
	2020-21	1.3	0.9–1.8	1.1 (HFB-1)
	2021-22	1.2	1.0–2.0	1.0 (HFB-1)
No. of seeds/pod	2019-20	2.9	2.3–3.8	3.1 (HFB-1)
	2020-21	2.9	2.2–3.8	3.3 (HFB-1)
	2021-22	3.0	2.4–4.0	2.6 (HFB-1)
Seed yield/plant (g)	2019-20	43.158	15.675–66.525	24.500 (Vikrant)
	2020-21	30.441	21.025–41.604	26.354 (Vikrant)
	2021-22	16.731	9.467–29.700	10.400 (HFB-1)
100-seed weight (g)	2019-20	69.474	41.854 - 111.253	31.927 (Vikrant)
	2020-21	60.777	39.2 00- 88.400	33.667 (Vikrant)
	2021-22	61.273	38.400 - 105.600	24.106 (Vikrant)
Pod yield/ plant (g)	2021-22	99.981	43.633 - 199.930	69.700 (HFB-1)

flowering and pod-filling period of March–May during 2021–22 than the other two years (Table 2). Under plain conditions faba bean can grow up to 30–35°C. However, in hilly areas, the temperature seems to be on lower side. Generally, the freezing winter in Kashmir arrests the growth of *rabi* crops and as soon as the temperature rises gradually from March onwards, the crops begin to grow rapidly. During these post-winter months, adequate rainfall is crucial for raising a good crop, especially in dryland areas. According to Flores *et al.* (2012), faba bean is a winter crop that grows better under cool and moist conditions, whereas hot and dry weather is unfavorable and could lead to decrease in the seed yield and quality. It has also been reported that the production response in faba beans is closely linked to the availability of water during the production phases and that heat stress during floral development reduced the seed yield (Giordano *et al.*, 1994, Abdelmula and Abuanja, 2007, Bishop *et al.*, 2016, Ton *et al.*, 2021).

Highly significant correlations were observed between pod length and plant height, pod width and pod length, seed yield and pod width, 100-seed weight with both pod length and pod width (Table 5). Similar correlations have been reported in some earlier studies on faba beans (Kumar *et al.*, 2017, Sharma *et al.*, 2017, Ton *et al.*, 2021).

To understand sources of variance among faba bean genotypes, principal component analysis (PCA) was carried out which grouped the 9 studied agro-morphological traits into the first three axes, describing more than 70%

Table 5: Pearson's correlation matrix for various agro-morphological traits of 39 local faba bean (*V. faba*) genotypes evaluated under rainfed conditions of Kashmir, India during three years of 2019-20, 2020-2021 and 2021-2022

	PH	*DTF*	*DTM*	*NB*	*PL*	*PW*	*SP*	*SY*	*SW*
PH	-								
DTF	0.015	-							
DTM	0.321 *	- 0.197	-						
NB	0.482 **	0.293	0.093						
PL	0.690 ***	-0.279	0.229	0.111	-				
PW	0.470 **	-0.127	0.183	0.032	0.774 ***	-			
SP	-0.082	0.294	-0.057	0.229	-0.127	-0.090	-		
SY	0.451 **	-0.189	0.191	0.072	0.465 **	0.548 ***	-0.152	-	
SW	0.346 *	-0.375 *	0.093	-0.249	0.738 ***	0.717 ***	-0.188	0.394 *	-

* p < .05, ** p < .01, *** p < .001

PH = Plant height, DTF = Days to 50% flowering, DTM = Days to 80% maturity, NB = Number of branches/plant, PL = Pod length, PW = Pod width, SP = Seeds/pod, SY = Seed yield/plant and SW = 100 - Seed weight

Table 6: Eigen values and percentage of total variance for PCA in 39 local faba bean (V. *faba*) genotypes evaluated under rainfed conditions of Kashmir, India during three years of 2019-20, 2020-2021 and 2021-2022

Trait	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*	*PC7*	*PC8*	*PC9*
Eigen value	3.498	1.759	1.061	0.835	0.638	0.602	0.314	0.185	0.108
Relative variance (%)	38.86	19.54	11.79	9.28	7.08	6.69	3.49	2.07	1.20
Cumulative variance (%)	38.86	58.4	70.19	79.47	86.55	93.24	96.73	98.8	100

Table 7: Vector loadings (varimax) for yield contributing traits in 39 local faba bean (*V. faba*) genotypes evaluated under rainfed conditions of Kashmir, India combined over three rabi seasons of 2019-20, 2020-2021 and 2021-2022

Trait	*Vec 1*	*Vec 2*	*Vec 3*	*Vec 4*	*Vec 5*	*Vec 6*	*Vec 7*	*Vec 8*	*Vec 9*
Plant height (cm)	0.381	0.392	-0.153	-0.190	-0.200	0.023	-0.599	-0.076	-0.484
Days to 50% flowering	-0.193	0.465	0.338	-0.203	0.302	0.664	-0.081	0.132	0.183
Days to 80% maturity	0.190	0.097	-0.718	0.478	0.179	0.389	0.098	0.101	0.074
No. of branches/plant	0.041	0.646	-0.153	-0.192	-0.267	-0.325	0.500	0.283	0.110
Pod length (cm)	0.488	0.051	0.127	0.048	-0.299	0.051	-0.128	-0.356	0.709
Pod width (cm)	0.455	0.020	0.290	0.074	0.124	0.199	0.563	-0.385	-0.425
No. of seeds/pod	-0.131	0.367	0.365	0.774	0.067	-0.276	-0.171	-0.027	-0.050
Seed yield/plant (g)	0.365	0.044	-0.017	-0.173	0.788	-0.406	-0.100	0.108	0.159
100-Seed weight (g)	0.427	-0.250	0.296	0.135	-0.181	0.128	-0.012	0.772	-0.038

Table 8: Mean and standard deviation for three clusters based on nine quantitative characters of 39 local faba bean (*V. faba*) genotypes evaluated under rainfed conditions of Kashmir, India combined over three *rabi* seasons of 2019-20, 2020-2021 and 2021-2022

Trait	*Cluster 1 (16 Genotypes)*	*Cluster 2 (15 Genotypes)*	*Cluster 3 (8 Genotypes)*
Plant height (cm)	70.6 ± 9.4	68.9 ± 5.4	74.8 ± 6.5
Days to 50% flowering	142.5 ± 1.2	143.9 ± 1.7	142.2 ± 1.6
Days to 80% maturity	196.2 ± 1.8	196.6 ± 1.5	197.4 ± 1.9
Number of branches/plant	2.3 ± 0.5	2.6 ± 0.4	2.2 ± 0.5
Pod length (cm)	7.3 ± 1.0	6.5 ± 0.7	8.5 ± 0.6
Pod width (cm)	1.4 ± 0.2	1.3 ± 0.2	1.7 ± 0.1
No. of seeds/pod	2.9 ± 0.2	3.0 ± 0.3	2.9 ± 0.3
Seed yield/plant (g)	29.745 ± 6.409	30.593 ± 4.789	36.211 ± 6.508
100 - Seed weight (g)	65.041 ± 5.363	47.811 ± 4.161	83.997 ± 4.039

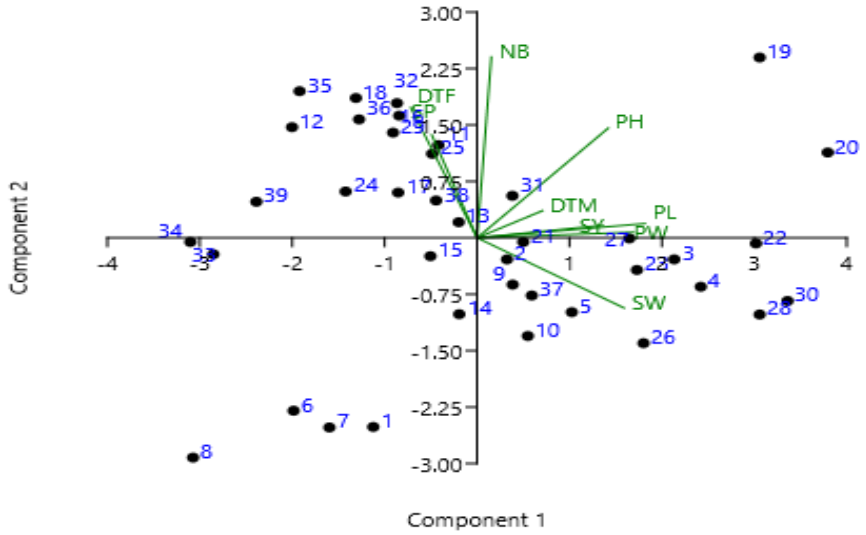


Figure 2: The first (X-axis) and second (Y-axis) principal component projection of 39 faba bean genotypes used in the present study. The dispersal of the 39 genotypes in the four quadrants of the biplot suggest appreciable genetic variability

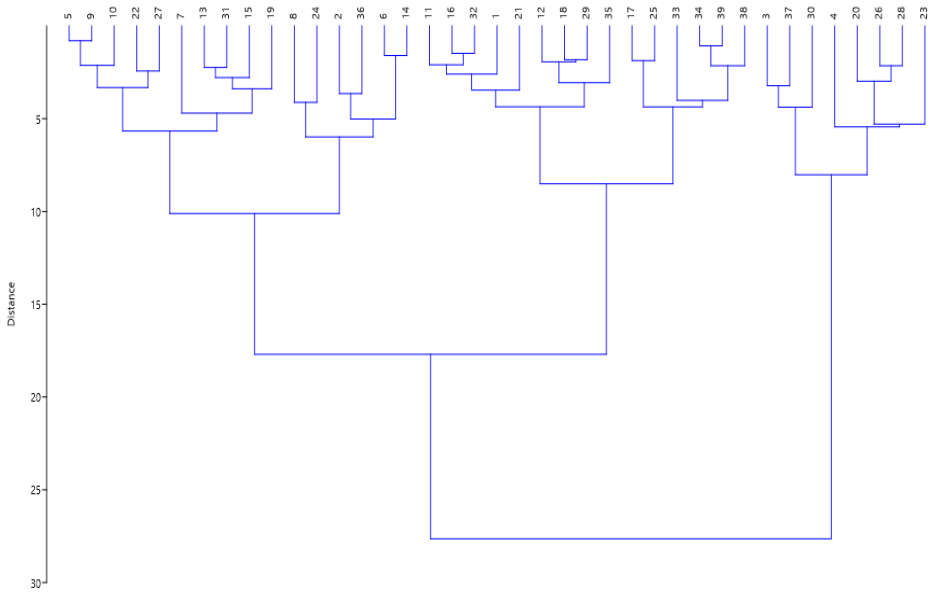


Figure 3: Unweighted pair group method with arithmetic average (UPGMA) cluster for the 39 faba bean genotypes based on 9 agronomic variables

of the total variations (Tables 6 and 7). The first principle component (PC1) demonstrated 38.86% of the total variation and was positively associated with pod length, pod width and 100-seed weight traits. Major effective traits in PC2 accounting for 19.54% variance were days to 50% flowering and no. of branches/plant, while PC3 demonstrated 11.79% variance and associated negatively with trait days to 80% maturity. The first PC thus, was more related to yield contributing traits while PC2 and PC3 solely to vegetative growth. Traits loading more on these first three principal components with Eigen values greater than 1 and contributing around 70% of the variance were used to cluster 39 faba bean genotypes into closely related groups. The critical examination of the dendrogram generated from an unweighted pair group method analysis (UPGMA) after the cluster analysis and PCA, revealed three clusters (Figures 2 and 3). The mean value ± SD of all the traits studied of these three clusters are presented in Table 8. Sixteen (16) genotypes representing 41% of the total genotypes grouped in cluster 1 with medium 100-seed weight. Cluster 2 with fifteen (15) genotypes has lower 100-seed weight and plant height. On the other hand cluster 3 with eight (8) genotypes recorded highest plant height, pod length, pod width, seed yield/plant and 100-seed weight. Average 100-seed weight of 65.041, 47.811 and 83.997 g was recorded for clusters 1, 2 and 3, respectively. Interestingly cluster 2 with lowest 100-seed weight also exhibited lowest average plant height of 68.9 cm, while cluster 3 with highest 100-seed weight recorded highest plant height as well. Average pod length and pod width was also lower in cluster 2. Short stem and smaller seeds are believed to be primitive characteristics and that small seed/pod features are said to be dominant over larger, more recent seed types (Cubero and Nadal, 2005). Broadly speaking our study has thus revealed the occurrence of three size classes of faba beans in Kashmir as three clusters differed appreciably in 100-seed weight. Thus, three distinct clusters were observed mainly on the basis of greater morphological similarity than place of collection in the present study as a result of the multivariate analysis. These findings corroborate earlier similar studies on faba beans (Duc *et al*., 2010, Chaeib *et al*., 2011, Laureles *et al*., 2019). According to Duc *et al.* (2010), the response of genotypes is differential since the adaptation to the environment and its interaction with it is determinant in a desirable phenotypic expression. Interestingly, all the 8 genotypes in cluster 3 are topmost in 100-seed weight among 39 genotypes studied. The average plant height, pod length and pod width besides 100-seed weight in this cluster are higher than the other two clusters, and these traits show strong positive correlations. Thus, while revealing good amount of variability in local faba bean collections, the present study provides an opportunity to select adapted genotypes with good yield potential traits.

Conclusion

The results of the current study showed high genetic diversity in faba bean genotypes collected from different areas of Kashmir. Significant variability has been recorded in traits of plant height, number of branches/plant, pod length, pod width, number of seeds/pod, seed yield/plant and 100-seed weight. Principal component and cluster analysis have revealed three distinct clusters, mainly on the basis of greater agro-morphological similarity. Genotypes with the highest average plant height, pod length, pod width, seed yield/plant and 100-seed weight were clustered in a separate group; all these traits showed significant positive correlations. Pod yield/plant evaluated during single year of 2021–22 also showed significant variation among the genotypes. These traits, therefore, should be given due consideration while performing selection for improving the performance of faba bean crop in the region. From the present study, it is also concluded that since the production response in faba beans is closely linked to the availability of water during the production phases, adequate rainfall during the months of active growth *i.e*., March-May in dryland areas of Kashmir is very crucial for raising a good faba bean crop. Thus, assured irrigation can enhance the overall performance of faba bean crop.

Acknowledgments

The authors thank the Director, ICAR-NBPGR New Delhi and the Head, Division of Germplasm Exploration and Collection, ICAR-NBPGR New Delhi for facilities and help.

References

Abdelmula AA and IK Abuanja (2007) Genotypic responses, yield stability, and association between characters among some of Sudanese faba bean (*Vicia faba* L.) genotypes under heat stress. *In*: Proceeding of the Conference on International Agricultural Research for Devolopment; Witzenhausen, Germany. pp 1-7.

Alghamdi SS, HM Migdadi, MH Ammar, JG Paull and KHM Siddique (2012) Faba bean genomics: Current status and future prospects. *Euphytica.* **186(3)**: 609-624.

Bishop J, SG Potts and HE Jones (2016) Susceptibility of faba bean (*Vicia faba* L.) to heat stress during floral development and anthesis. *J. Agron. Crop Sci.* **202**: 508-517. *doi: 10.1111/jac.12172*

Caracuta V, M Weinstein-Evron, D Kaufman, R Yeshurun, J Silvent and E Boaretto (2016) 14,000-year-old seeds indicate the Levantine origin of the lost progenitor of faba bean. *Sci Rep* **6**: 37399.

Chaieb N, B Mohamed and M Mars (2011) Growth and yield parameters variability among faba bean (*Vicia faba* L.) genotypes. *J. Natural Products Plant Res* **1(2)**: 81-90.

Cubero JI (1974) on the evolution of *Vicia* faba L. *Theor Appl.* **45(2)**: 47-51.

Cubero JI (2011) The faba bean: A historic perspective. *Grain Legum* **56**: 5-7.

Cubero JI and S Nadal (2005) Faba bean (*Vicia faba* L.), *In*: Singh RJ and PP Jauhar (Eds.) Genetic Resources, Chromosome

Engineering and Crop Improvement. Vol 1, Grain legumes. pp 163-186.

Dhull SB, MK Kidwai, R Noor, P Chawla and PK Rose (2021) A review of nutritional profile and processing of faba bean (*Vicia faba* L.). *Legume Science*, e129. https://doi.org/10.1002/leg3.129

Duc G (1997) Faba bean (*Vicia faba* L.). *Field Crops Res* **53(1-3)**: 99-109.

Duc G, JM Aleksić, P Marget, A Mikic, J Paull, RJ Redden, O Sass, FL Stoddard, A Vandenberg, M Vishnyakova and AM Torres (2015) Faba bean. *In* A De Ron (Ed.), *Grain Legumes. Handbook of Plant Breeding*, vol. 10: pp. 141-178. Springer, New York, NY, USA. *https://doi.org/10.1007/978-1-4939-2797-5_5*

Duc G, S Bao, M Baum, B Redden, M Sadiki, MJ Suso, M Vishniakova and X Zong (2010) Diversity maintenance and use of *Vicia faba* L. genetic resources. *Field Crop Res* **115(3)**: 270-278.

Elshafei AAM, MA Amer, MAM Elenany and AGA and E Helal (2019) Evaluation of the genetic variability of faba bean (*Vicia faba* L.) genotypes using agronomic traits and molecular markers. *Bulletin of the National Research Centre* **43**: 106. *https://doi.org/10.1186/s42269-019-0145-3*

FAOSTAT (2021) Database, available online: www.faostat.fao.org

Flores F, M Hybl, J Knudsen, P Marget, F Muel, S Nadal, L Narits, B Raffiot, O Sass and I Solis (2013) Adaptation of spring faba bean types across European climates. *Field Crop Res* **145**: 1-9.

Giordano I, V Abbate, A Ierna, MG Lombardo, A Accardo, G Amato, D Gianbalvo, D Gristina, R D'Amore and R Ferrari (1994) Potenzialità produttiva del cece in differenti condizioni ambientali. *Agric Ric* **155**: 95-104.

Hauggaard-Nielsen H, S Mundus and ES Jensen (2009) Nitrogen dynamics following grain legumes and subsequent catch crops and the effects on succeeding cereal crops. *Nutr Cycl Agroecosys* **84**: 281-291.

Kumar P, R Das, S Bishnoi and S Vinay (2017) Inter-correlation and path analysis in faba bean (*Vicia faba* L.). *Electronic Journal of Plant Breeding* **8**: 395-397. *doi: 10.5958/0975-928x.2017.00059.x*

Ladizinsky G (1998) Plant evolution under domestication. Kluwer Academic Publishers, Dortrecht, Netherlands.

Laureles MES, D de Jesús P López, AG Huerta and LM Vázquez García (2019) Phenotypic variability in faba beans collections from the Valley Toluca-Atlacomulco, Mexico. *Revista Mexicana de Ciencias Agrícolas* **10(3)**: 713-727.

Martineau-Côté, D, A Achouri, S Karboune and LL Hocine (2022) Faba Bean: An Untapped Source of Quality Plant Proteins and Bioactives. *Nutrients* **14(8)**: 1541. *doi: 10.3390/nu14081541*

Mínguez MI and D Rubiales (2021) Faba bean. *In* Sadras VO and DF Calderini (Eds.), Crop Physiology Case Histories for Major Crops (pp. 452-481). Academic Press. *https://doi.org/10.1016/B978-0-12-819194-1.00015-3*

Musallam IW, GN Al-Karaki, KI Ereifei, A Rahman and ARM Tawaha (2004) Chemical composition of faba bean genotypes under rainfed and irrigation conditions. *Int J Agr Biol* **6**: 359-362.

Nadal S, MJ Suso and MT Moreno (2003) Management of *Vicia* faba genetic resources: Changes associated to the selfing process in the major, equina and minor groups. *Genet Resour Crop Evol* **50**: 183-192.

Phillips DA (1980) Efficiency of symbiotic nitrogen-fixation in legumes. *Annu Rev Plant Physiol Plant Mol Biol* **31**: 29-49.

Rahate KA, M Madhumita and PK Prabhakar (2020) Nutritional composition, anti-nutritional factors, pre-treatments-cum-processing impact and food formulation potential of faba bean (*Vicia faba* L.): A comprehensive review. *LWT - Food Science and Technology* **138**: 110796.

Revilla I (2015) Impact of thermal processing on faba bean (*Vicia faba*) composition. Processing and impact on active components in food. Academic Press, doi: *https://doi.org/10.1016/B978-0-12-404699-3.00040-8*

Serradilla JM, T De Mora and MT Moreno (1993) Geographic dispersion and varietal diversity in *Vicia* faba L. *Genet Res Crop Evol* **40**: 143-151.

Sharma PP, M Vyas and DR Meghawal (2017) Estimation of genetic variability and correlation analysis in field pea (*Pisum sativum* L.) genotypes. *J. plant dev. sci.* **9**: 53-56.

Singh AK and BP Bhatt (2012) Faba bean: unique germplasm explored and identified. *Hort Flora Res Spectrum* **1**: 267-269.

Singh AK, BP Bhat, PK Sundaram, N Chndra, RC Bharati and SK Patel (2012) Faba bean (*Vicia faba* L.) phenology and performance in response to its seed size class and planting depth. *Int J Agric Stat Sci* **8**: 97-109.

Tanno K and G Willcox (2006) The origins of cultivation of *Cicer arietinum* L. and *Vicia* faba L.: Early finds from Tell el-Kerkh, northwest Syria, late 10th millennium b.p. *Veg Hist Archaeobot* **15(3)**: 197-204.

Ton A, T Karakoy, AE Anlarsal and M Turkeri (2019) Genetic diversity for agro-morphological characters and nutritional compositions of some local faba bean **(***Vicia faba* L.) genotypes. *Turk J Agric For* **45**: 301-312, *doi:10.3906/tar-2008-74*

USDA (2021) Food Data Central (Nutrient Database). https://fdc.nal.usda.gov/

Valen IM, MR Maia, N Malushi, HM Oliveira, L Papa, JA Rodrigues, AJ Fonseca and AR Cabrita (2018) Profiling of phenolic compounds and antioxidant properties of European varieties and cultivars of *Vicia* faba L. pods. *Phytochemistry* **152**: 223-229. *https://doi.org/10.1016/j.phytochem.2018.05.011*

Watson CA, M Reckling, S Preissel, J Bachinger, G Bergkvist, T Kuhlman, K Lindström, T Nemecek, CFE Topp, A Vanhatalo, P Zander, D Murphy-Bokern and FL Stoddard (2017) Grain legume production and use in European agricultural systems. *Advances in Agronomy* **144**: 235-303. https://doi.org/10.1016/bs.agron.2017.03.003

Ye Y, L Lang, M Xia and J Tu (2003) *Faba* Beans in China. China Agriculture Press, Beijing, China.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.03

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Genetics of Yield and Fruit Quality Traits in Bitter Gourd (*Momordica charantia* L.)

Vinay N. D[1], Asima Aamin[1,4], Tusar K. Behera[1, 3*], Anilabh D. Munshi[1], Gograj S. Jat[1], Shyam S. Dey[1] and Ranjith K. Ellur[2]

Abstract

Utilization of genetic resources in crop breeding requires a basic understanding of the economic traits they are recognized for. Determination of genetics of traits helps to decide the appropriate breeding strategy for developing improved varieties. Segregating populations developed using *Momordica charantia* var. *charantia* and *M. charantia* var. *muricate* genotypes were used for generation mean analysis (GMA) to work out the gene effects controlling yield and fruit quality traits in bitter gourd. The study confirmed the role of non-allelic interactions and predominance of dominance gene effect (h) for most economic traits, which indicates the suitability of recurrent selection or heterosis breeding for the improvement of these traits. Segregation analysis indicated the single dominant gene control of fruit epicarp color, tubercle nature and fruit ridges. The predominance of complementary type of epistasis for most traits implies the genetic divergence between the parents used and the possibility of realizing a greater genetic gain in the breeding programme using these genetic resources.

Keywords: Dominance effect, Epistasis interaction, Generation mean analysis, Genetic studies, *Momordica charantia*.

[1]Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, India.

[2]Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India.

[3]ICAR-Indian Institute of vegetable Research, Varanasi, Uttar Pradesh, India.

[4]Directorate of Extension, Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir, India.

***Author for correspondence:**

tusar@rediffmail.com

Received: 23/08/2023 **Revised:** 12/10/2023

Accepted: 13/10/2023

How to cite this article: Vinay, N.D., Aamin, A., Behera, T.K., Munshi, A.D., Jat, G.S., Dey, S.S., Ellur, R.K. (2023). Genetics of Yield and Fruit Quality Traits in Bitter Gourd (*Momordica charantia* L.). Indian J. Plant Genetic Resources. 36(3), 356-361. **DOI:** 10.61949/0976-1926.2023.v36i03.03

Introduction

Momordica charantia L. (Syn. bitter gourd, balsam pear) (2n = 22) is a traditional vegetable and medicinal crop of tropics and subtropics of Asia, South America and East Africa (Heiser, 2016). Bitter gourd belongs to Cucurbitaceae family and is known for its distinctive bitter flavor and high nutritional value, specifically ascorbic acid and iron (Behera, 2004). As a medicinal crop, it is widely known for its anti-diabetic, anti-HIV and anti-tumor properties (Behera *et al*., 2008). Bitter gourd is a highly cross-pollinated crop due to its monoecious sex form, which results in wide variability. Mainly, Indian bitter gourd gene pool exhibits high morphological variation in growth habit, maturity, fruit shape, size, color, surface texture and sex expression (Behera *et al*., 2006). This genetic variability is vital in developing varieties as per consumer demand. Market preference for bitter gourd varieties varies from region to region with respect to its fruit quality traits, such as fruit color, length, shape, and tubercles (Rathod *et al*., 2019). For instance, dark green to glossy green fruits are preferred in northern India. White fruits are ideal in the south Indian market, whereas in eastern parts of the country, small and dark green fruits are desired (Behera *et al*., 2010). Hence to exploit the existing genetic variation in order to develop varieties suitable for various market segments, elucidating genetics of these traits is crucial. To successfully determine trait genetics, populations derived from diverse parents should be used for genetic analysis. The generated knowledge through this

Table 1: Generation means for earliness and yield traits in cross PVGy-201 × PDM and CBM-12 × DBG-4 of bitter gourd

Traits	*Cross*	*P1*	*P2*	*F1*	*F2*	*B1*	*B2*
Node to first female flower	PVGy-201 × PDM	3.05 ± 0.29	11.400 ± 0.72	5.62 ± 0.36	6.57 ± 0.16	4.83 ± 0.23	9.78 ± 0.31
	CBM-12 × DBG-4	12.60 ± 0.77	7.400 ± 0.64	7.82 ± 0.39	7.95 ± 0.40	9.91 ± 0.26	6.01 ± 0.28
Days to first female flower	PVGy-201 × PDM	26.80 ± 0.57	45.250 ± 1.01	32.10 ± 0.38	35.17 ± 0.46	30.36 ± 0.32	36.90 ± 4.33
	CBM-12 × DBG-4	79.45 ± 1.08	78.450 ± 1.60	63.450 ± 1.60	71.34 ± 0.68	69.46 ± 0.48	66.33 ± 0.74
Node to first male flower	PVGy-201 × PDM	-	-	-	-	-	-
	CBM-12 × DBG-4	9.05 ± 0.39	4.15 ± 0.38	4.45 ± 0.26	7.12 ± 0.33	6.30 ± 0.20	4.16 ± 0.17
Days to first male flower	PVGy-201 × PDM	-	-	-	-	-	-
	CBM-12 × DBG-4	64.20 ± 1.34	65.95 ± 1.39	55.82 ± 0.89	68.67 ± 0.80	61.66 ± 0.62	66.33 ± 0.74
Fruit length (mm)	PVGy-201 × PDM	113.87 ± 3.30	180.39 ± 2.27	165.48 ± 1.78	144.30 ± 2.71	140.53 ± 1.42	170.78 ± 1.39
	CBM-12 × DBG-4	54.19 ± 1.16	165.35 ± 1.90	109 ± 0.90	99.52 ± 1.79	73.01 ± 0.83	128.62 ± 0.91
Fruit weight (gm)	PVGy-201 × PDM	89.47 ± 4.26	145.27 ± 2.89	139.84 ± 1.352	120.46 ± 2.30	128.29 ± 1.12	147.39 ± 1.15
	CBM-12 × DBG-4	17.38 ± 0.68	96.85 ± 1.79	56.65 ± 0.93	47.32 ± 1.65	29.89 ± 0.60	70.25 ± 0.92
Fruit diameter (mm)	PVGy-201 × PDM	42.82 ± 0.81	38.97 ± 0.72	44.46 ± 0.57	40.79 ± 0.35	42.91 ± 0.45	38.06 ± 0.46
	CBM-12 × DBG-4	34.56 ± 0.94	41.55 ± 0.91	38.12 ± 0.44	38.49 ± 0.53	34.83 ± 0.35	39.483 ± 0.29
Number of fruits per plant	PVGy-201 × PDM	20.70 ± 0.84	13.20 ± 0.56	22.52 ± 0.64	19.01 ± 0.45	23.95 ± 0.51	18.05 ± 0.57
	CBM-12 × DBG-4	24.30 ± 1.54	13.50 ± 1.03	25.37 ± 0.87	18.47 ± 0.50	19.85 ± 0.61	16.33 ± 0.43
Fruit yield per plant (gm)	PVGy-201 × PDM	1,505.0 ± 100.9	1,775.7 ± 97.9	2,746.5 ± 94.1	1,556.2 ± 61.2	2,682.7 ± 74.9	2,261.1 ± 84.1
	CBM-12 × DBG-4	431.7 ± 35.4	1,308.5 ± 105.7	1,427.7 ± 43.8	843.5 ± 35.8	682.9 ± 22.5	1,288.1 ± 38.3

Table 2: Test of significance of A, B, C, and D scales earliness and yield traits in cross PVGy-201 × PDM and CBM-12 × DBG-4 of bitter gourd

Traits	*Cross*	*A*	*B*	*C*	*D*
Node to first female flower	PVGy-201 × PDM	-1.09* ± 0.46	-2.54* ± 0.72	3.39* ± 0.88	-3.51** ± 0.36
	CBM-12 × DBG-4	0.59 ± 0.72	3.19* ± 0.66	4.2* ± 1.447	-0.22 ± 0.62
Days to first female flower	PVGy-201 × PDM	-1.83* ± 0.67	3.55* ± 0.98	-4.468* ± 1.635	3.09** ± 0.75
	CBM-12 × DBG-4	3.96* ± 1.53	9.23** ± 1.91	-0.57 ± 3.278	6.88** ± 1.15
Node to first male flower	PVGy-201 × PDM	-	-	-	-
	CBM-12 × DBG-4	0.90* ± 0.44	0.26 ± 0.41	-6.39** ± 1.08	3.77** ± 0.50
Days to first male flower	PVGy-201 × PDM	-	-		-
	CBM-12 × DBG-4	-3.30* ± 1.44	-10.89** ± 1.57	-32.90** ± 2.93	9.35** ± 7.06
Fruit length (mm)	PVGy-201 × PDM	-1.71 ± 3.33	4.302 ± 2.84	48.02** ± 8.57	-22.71** ± 4.09
	CBM-12 × DBG-4	6.20** ± 1.59	6.15* ± 1.99	17.56* ± 5.49	-2.60 ± 2.68
Fruit weight (gm)	PVGy-201 × PDM	-27.26** ± 3.53	-9.67* ± 2.79	32.56** ± 7.71	-34.75** ± 3.45
	CBM-12 × DBG-4	4.25** ± 1.18	2.98 ± 1.94	18.23** ± 5.04	-5.49* ± 2.46
Fruit diameter (mm)	PVGy-201 × PDM	-1.53 ± 0.95	4.31** ± 0.92	1.54 ± 1.50	0.62 ± .067
	CBM-12 × DBG-4	3.01* ± 0.89	0.71 ± 0.832	-1.61 ± 1.87	2.67* ± 0.82
Number of fruits per plant	PVGy-201 × PDM	-4.67** ± 1.04	-0.37 ± 1.01	2.89 ± 1.73	-3.97** ± 0.84
	CBM-12 ×DBG-4	7.97** ± 1.52	4.20** ± 1.13	10.64** ± 2.29	0.768 ± 0.88
Fruit yield per plant (gm)	PVGy-201 × PDM	-1,113.8** ± 144.0	-0.14 ± 152.9	2,548.6** ± 239.9	-1,831.3** ± 117.6
	CBM-12 × DBG-4	293.66** ± 5.75	-40.06 ± 0.41	821.63** ± 5.76	-284.01** ± 4.7

(*, ** indicated significant effect at p = 5 and 1%)

study will help in better utilization of these germplasm in crop improvement through appropriate breeding methods.

Momordica charantia is the most widely grown species among the seven *Momordica* species found in India (Joseph & Antony 2010). Bitter gourd cultivars in India fall into two botanical groups, *M. charantia var. charantia*, which produces large fusiform fruits and *M. charantia var. muricata* (wild), which bears small and disc-shaped fruit with numerous tubercles on its surface (Chakravarty 1990). These two recognized botanical varieties show striking morphological

Table 3: Estimates of genetic effects for earliness and yield traits in cross PVGy-201 × PDM and CBM-12 × DBG-4 of bitter gourd

Traits	*Cross*	[m]	[d]	[h]	[i]	[j]	[l]	*χ2*	*Epistasis*
Node to first female flower	PVGy-201 × PDM	5.57** ± 0.11	4.90** ± 0.278	5.42** ± 0.818	7.02** ± 0.725	-1.45 ± 0.784	-10.65** ± 1.423	94.37**	D
	CBM-12 × DBG-4	7.85** ± 0.283	3.90** ± 0.274	-1.72 ± 1.335	0.45 ± 1.256	2.60* ± 0.897	3.32** ± 1.815	24.72*	C
Days to first female flower	PVGy-201 × PDM	35.17** ± 0.325	-6.53** ± 0.382	-10.11** ± 1.588	-6.18** ± 1.509	5.38** ± 1.122	7.90* ± 2.236	38.69**	D
	CBM-12 × DBG-4	71.34** ± 0.484	3.13** ± 0.628	-29.27** ± 2.659	-13.77** ± 2.306	5.26* ± 1.857	26.97** ± 4.129	47.61**	C
Node to first male flower	PVGy-201 × PDM	-	-	-	-	-	-	-	-
	CBM-12 × DBG-4	7.12** ± 0.234	2.13** ± 0.188	-9.70*8 ± 1.043	-7.55** ± 1.008	-0.63 ± 0.541	8.72** ± 1.317	57.01**	C
Days to first male flower	PVGy-201 × PDM	-	-	-	-	-	-	-	-
	CBM-12 × DBG-4	68.67** ± 0.565	-4.66** ± 0.688	-27.95*8 ± 2.807	-18.70** ± 2.647	-7.58* ± 1.943	4.50 ± 4.022	140.0**	-
Fruit length (mm)	PVGy-201 ×PDM	144.30** ± 1.92	-30.25** ± 1.40	63.77** ± 8.40	45.43** ± 8.18	6.01 ± 4.0	-42.84** ± 10.25	36.96**	C
	CBM-12 × DBG-4	99.52** ± 1.268	-55.60** ± 0.873	-6.52* ± 5.465	5.20 ± 5.362	-0.04 ± 2.355	7.16** ± 6.506	25.38**	D
Fruit weight (gm)	PVGy-201 × PDM	120.46** ± 1.63	-19.10** ± 1.140	91.98** ± 7.213	69.51** ± 6.913	17.59** ± 4.299	-106.45** ± 8.96	148.4**	C
	CBM-12 × DBG-4	47.32** ± 1.169	-40.36** ± 0.784	0.52 ± 5.024	10.99* ± 4.934	-1.26 ± 2.076	-3.75 ± 5.942	21.92**	-
Fruit diameter (mm)	PVGy-201 × PDM	40.79** ± 0.249	4.85** ± 0.457	-0.67 ± 1.463	-1.24 ± 1.351	5.85** ± 1.195	4.02 ± 2.363	28.30**	-
	CBM-12 × DBG-4	38.49** ± 0.377	-4.64* ± 0.326	-5.28** ± 1.737	-5.34** ± 1.644	-2.30* ± 1.136	9.072** ± 2.288	18.84**	D
Number of fruits per plant	PVGy-201 × PDM	19.01** ± 0.324	5.90** ± 0.549	13.52** ± 1.794	7.94** ± 1.699	4.30* ± 1.311	12.99** ± 2.799	35.57**	C
	CBM-12 × DBG-4	18.47** ± 0.355	3.51** ± 0.534	2.94 ± 1.992	-1.53 ± 1.776	-3.76* ± 1.695	13.71** ± 3.135	35.11**	C
Fruit yield pe plant (gm)	PVGy-201 × PDM	1,556.2** ± 43	421.5** ± 79.6	4,768.7** ± 249	3,662.6** ± 235	1,113.7** ± 187	4,776.6** ± 398	297.5**	C
	CBM-12 × DBG-4	843.53** ± 25.3	-605.2** ± 31.4	925.6** ± 129.4	568.0** ± 119.3	-333.7* ± 100.8	314.4** ± 190.1	66.82**	C

(*, ** indicated significant effect at p = 5 and 1%)

differences and exhibit remarkable diversity for economic traits. Hence, using these two botanical types in breeding programs is essential for effective bitter gourd improvement.

Yield is a complex polygenic trait, determined by the cumulative effects of several yield-contributing traits such as fruit length, weight, number of female flowers, etc. Earliness is another important trait that is directly correlated with yield. Farmers prefer early varieties due to its suitability to multiple cropping systems and ability to fetch high prices in the early season market (Dey *et al*., 2012). To breed high-yielding early varieties, basic understanding of the genetics of component traits and knowledge of gene action/effects functioning in a particular breeding population is essential (Vinay *et al*., 2023). Keeping above facts in mind, current generation mean analysis study was conducted to estimate the various genetic components of economic traits in bitter gourd using population derived from genetically diverse parental lines. The results of this study will lay the foundation for development of novel early-high yielding varieties and hybrids of bitter gourd to boost the production.

Material and Methods

Present investigation was conducted at experimental field of ICAR-Indian Agricultural Research Institute, New Delhi, India. For genetic analysis, two cross combinations PVGy-1 × Pusa Do Mausami (PDM) and CBM-12 × DBG-4, were used to develop populations (F1, F2, B1 and B). PVGy1, PDM and DBG-4 lines belong to *M. charantia* var *charantia* where as CBM-12 belongs to *M. charantia* var *muricata* group. The F_1 progenies were developed during rainy season (June-October, 2018), F_2 and backcross progenies (B_1 and B_2) were genertaed from F_1 plants during the spring-summer of 2019 under the insect-proof net house. Final trial for evaluation was laid out with six generations (P_1, P_2, F_1, F_2, B_1 and B_2) under natural/open field conditions during August- October of 2019 in a completely randomized block design with three replications. The data was recorded from 10 plants in each parent, 20 plants of F_1, 30 plants of B_1 and B_2 generations and 118 (PVGy-1 × Pusa Do Mausami) and 102 (CBM-12 × DBG-4) plants in F_2 progenies. The phenotypic observations were recorded for four earliness-related traits, namely, node to first female flower (NFFF), days to first female flower (DFFF), node to first male flower (NFMF) and days to first male flower (DFMF) and five yield traits *viz*. namely, fruit length (cm), fruit diameter (cm), fruit weight (g), number of fruits per plant and fruit yield/plant (g). Two male flower-related parameters (NFMF, DFMF) were not considered in first cross (PVGy-1 × PDM) since the female parent (PVGy-1) was a gynioecious line. Spacing, nutrition, irrigation, cultural practices and plant protection measures were followed as per recommendations to raise a successful crop in the experimental plots.

Generation mean analysis (GMA) was performed as suggested by Hayman (1958) to estimate genetic

Table 4: Inheritance pattern of fruit epicarp color, tubercle nature and ridgeness in bitter gourd in PVGy-201 × PDM and CBM-12 × DBG-4 of bitter gourd

Fruit epicarp colour in cross - CBM-12 × DBG-4						
Population	*Total*	*Observed*		*Expected ratio*	*χ2 value*	*p-value*
	Green fruits	White fruits				
P1(CDM-12)	10	10	-	-		
P2 (DBG-4)	10	-	10	-		
F1(CBM-12 × DBG-4)	20	20	-	-		
F2	102	80	22	3:1	0.64	0.43
B1(F1 × CBM-12)	30	30	-	-		
B2(F1 × DBG-4)	30	17	13	1:1	0.53	0.47
Fruit tubercle nature in cross - CBM-12 × DBG-4						
Population	*Total*	*Observed*		*Expected ratio*	*χ2 value*	*p-value*
	Conspicuous	Non-conspicuous				
P1(CDM-12)	10	10	-	-		
P2 (DBG-4)	10	-	10	-		
F1(CBM-12 × DBG-4)	20	20	-	-		
F2	102	74	28	3:1	0.33	0.57
B1(F1 × CBM-12)	30	30	-	-		
B2(F1 × DBG-4)	30	16	14	1:1	0.13	0.72
Fruit ridgeness in cross - PVGy-201 × Pusa Do Mausami (PDM)						
Population	*Total*	*Observed*		*Expected ratio*	*χ2 value*	*p-value*
	Dis-continuous	Continuous				
P1(PVGy-201)	10	10	-	-		
P2 (PDM)	10	-	10	-		
F1(PVGy-1 × PDM)	20	20	-	-		
F2	118	84	34	3:1	0.92	0.34
B1(F1 × PVGy- 201)	30	17	13	1:1	0.53	0.47
B2(F1 × PDM)	30	30	-	-		

components. At first, using means of different generations, ABCD scales (Hayman and Mather, 1955) were tested to check for the presence of non-allelic interactions (epistasis) and then the data was subjected to joint scaling test which integrates multiple scaling tests to examine the competence of simple additive–dominance model or to detect epistasis using χ2 test. The six-parameter or di-genic interaction model (Hayman, 1958) was used to estimate the gene effects whenever the χ2 and ABCD scaling test is inadequate. These parameters represent mean effect [m], genetic effects including additive [d] and dominance [h], and gene interaction effects comprising additive × additive [i], additive × dominance [j] and dominance × dominance [l]. Student 't' test was used to declare the significance of the estimated scales (ABCD) and gene effects (m, d, h, i, j, l). The type of epistasis was determined as per Kearsey and Pooni, 1996.

To study the genetic inheritance of fruit traits *viz*. fruit epicarp color, ridgeness, tubercle nature, chi-square (χ2) test suggested by Panse and Sukhatme (1985) was done. These traits are expected as controlled by single gene.

Results and Discussion

The mean values (Table 1) of F_1 for fruit length, fruit weight and fruit diameter (in CBM-12 × DBG-4) were greater than the mid-parent values (average of the two parents) which indicate average heterosis, whereas F_1 mean values of number of fruits per plant and yield per plant and fruit diameter (in PVGy-201 × PDM) was higher than better parent values which indicated over dominance/ heterobeltotis. Similarly, for earliness-related traits, in both cross combinations mean values of F_1 implied negative average heterosis which is desirable. These results suggest the scope for improvement yield and earliness through heterosis breeding. There was decline in mean values of yield traits of F_2 population than corresponding F_1 in all yield traits. This apparently indicated influence of inbreeding depression. These findings are in agreement with the results obtained by Dey *et al*. (2012) in bitter gourd. The mean effect (m) was significant for all studied traits among three crosses, indicated that the traits were quantitatively inherited.

Significance of one or more scales (A, B, C, D) as shown in Table 2 and joint scaling χ2 (Table 3) test for all the traits under study in both crosses implies presence of digenic non-allelic interaction (epistasis). Hence, the simple additive-dominance model cannot satisfactorily explain the genetics of these traits. Therefore, according to Jinks and Jones (1958), the six-parameter model was employed to estimate six components of genetic variation, *viz*. m, d, h, i, j and l. The presence of non-allelic gene interactions involved in genetic control of quantitative yield traits in bitter gourd was previously reported by Kumari *et al*. (2015).

Higher magnitude and highly significant values of dominance effect (h) in desired direction was obtained for earliness-related traits like days to first female flower (both cross), node to first male flower (CBM12 × DBG-4) and day to first male flower (CBM12 × DBG-4). Additive × additive (i) type of non-allelic interaction was highly significant and negative for these traits. Similarly, higher magnitude significant-positive values of 'h' was observed for major yield parameters, number of fruits per plant and fruit yield per plant in both crosses, which indicates predominance of dominant gene effect. To add to this, of the three non-allelic interactions dominance × dominance (l) gene interaction was found to be positive and significant for these yield related traits. Hence, while breeding for improvement of earliness and yield traits selection should be delayed until heterozygosity is reduced in population. According to result of our study, hybrid development through heterosis breeding would be the most appropriate breeding method to evolve early and high-yielding hybrids using these cross combinations. For fruit length and fruit weight traits in cross PVGy-201 × PDM, additive effect (d) was positively significant with higher magnitude than dominance effect (h). In addition to this, additive × additive (i) type of non-allelic interactions was found to be highly significant. This clearly implies the suitability of simple selection procedure for the improvement of these traits. Complementary epistasis was observed for most earliness and yield traits under study, which implies that parents selected for crossing were diverse for that particular trait and desirable alleles for the traits are distributed among the parents. Hence, it is possible to realize greater genetic gain in a breeding programme. Our results are supported by the findings of Dey *et al*. (2012) in Bitter gourd.

The inheritance pattern of fruit epicarp color and tubercle nature was studied using cross of CBM-12 (green fruits with conspicuous tubercle) × DBGS-4 (white fruit with non-conspicuous tubercle). The analyzed result presented in Table 4 implies monogenic nature of both traits. Green fruit epicarp color is dominant over white, and conspicuous tubercles nature was dominant over non-conspicuous. Similar monogenic dominant nature of inheritance of fruit color was earlier reported by Hu *et al.* (2002) in bitter gourd. Our result of genetics of tubercle nature is supported by earlier studies (Rao *et al*., 2022). Phenotypic data obtained from cross PVGy-201 (dis-continuous) × PDM (continuous) was utilized to study the genetics of fruit ridgeness. The segregation pattern of fruit ridgeness in filial and backcross generations supported the single dominant gene hypothesis of genetic control. Discontinuous ridgeness was dominant over continuous ridgeness. Our study's result is supported by Rao *et al*.'s findings, 2022.

Conclusion

The genetic analysis for estimation of gene effects controlling economic traits in bitter gourd was done using diverse parental lines. Both additive and non-additive gene interactions control the economically important earliness and yield traits in bitter gourd. Therefore, recombination breeding with selection in later generations would be the most suitable way to improve these quantitative traits. Simple selection can give desired results when additive gene action is predominant. At the same time, dominant gene effects should be concentrated for better results. The traits governed by additive gene effect can be effectively improved through pedigree method. On the other hand, traits regulated by non-additive gene interaction (dominance effect) could be improved by heterosis breeding and through recurrent selection for specific combining ability. The traits with high magnitude of dominance and additives gene effects can be improved by pedigree or bulk methods or reciprocal recurrent selection methods. From the present study and most of the earlier reports, it is now clear that the fruit quality traits (color, tubercles and ridgeness) are qualitatively inherited *i.e.* by single dominant gene. Simple genetics of these fruit traits will permit the bitter gourd breeder to incorporate these traits in hybrids. The predominance of complementary epistasis for most economic traits studied implies the genetic divergence between the parents used and the possibility to realize greater genetic gain in breeding programme using these genetic resources. The study also implies the importance of populations derived from diverse genetic resources for the effective genetic analysis of phenotypic expressions in bitter gourd.

References

Behera TK (2004) Heterosis in bitter gourd. In: Singh PK, Dasgupta SK and Tripathi SK (ed) The Haworth Press. New York, USA, pp 217-221.

Behera TK, Behera S, Bharathi LK, John KJ, Simon PW and Staub JE (2010) Bitter gourd: botany, horticulture, Breeding. *Hortic. Rev.* **37**: 101.

Behera TK, Staub JE, Behera S and Simon PW (2008) Bitter gourd and human health. *Med. Aromat. Plant Sci. Biotechnol.* **1(2)**: 224-226.

Behera TK. Dey SS, and Sirohi PS (2006) DBGy-201and DBGy-202: two gynoecious lines in bitter gourd (*Momordica charantia* L.) isolated from indigenous source. *Indian. J. Genet. Plant.*

Breed. **66**: 61–62.

Chakravarty HL (1990) Cucurbits of India and their role in the development of vegetable crops In: Bates DM, Robinson RW and Jeffrey C (ed) Biology and Utilization Of Cucurbitaceae Cornell University Press, Ithaca, New York, pp 325-34.

Dey SS, Behera TK, Munshi AD, Rakshit S and Bhatia R (2012) Utility of gynoecious sex form in heterosis breeding of bitter gourd and genetics of associated vegetative and flowering traits. *Indian J. Hortic.* **69(4)**: 523–29.

Hayman BI (1958) The separation of epistatic from additive and dominance variation in generation means. *Heredity,* **12**: 371–90.

Hayman BI (1960) Maximum likelihood estimation of genetic components of variation. *Biometrics,* **16(3)**: 369-381.

Hayman BI, Mather K (1955) The description of genetics of interaction in continuous variation. *Biometrics*, **51**: 69–82.

Heiser CB (2016) The gourd book. University of Oklahoma Press, 248p.

Hu KL, Fu QM and Wang GP (2002) Study on the heredity of fruit color of *Momordica charantia*. *China Veg.* 11– 12.

Jinks JL, Jones RM (1958) Estimation of the components of heterosis. *Genetics,* **43**: 223-34.

Joseph JK and Antony VT (2010) A taxonomic revision of the genus *Momordica* L. (Cucurbitaceae) in India. *Indian J, Plant Genet. Resour.* **23**: 172–184.

Kearse MJ and Pooni HS (1996) *The genetic analysis of quantitative traits*, Chapman and Hall, London.

Kumari M, Behera TK, Munshi AD and Talukadar A (2015) Inheritance of fruit traits and generation mean analysis for estimation of horticultural traits in bitter gourd. *Indian. J. Hortic.* **72(1)**: 43–48.

ND, Vinay., Matsumura, H., Munshi, A.D., Ellur, R.K., Chinnusamy, V., Singh, A., Iquebal, M.A., Jaiswal, S., Jat, G.S., Panigrahi, I. and Gaikwad, A.B., (2023). "Molecular mapping of genomic regions and identification of possible candidate genes associated with gynoecious sex expression in bitter gourd. Frontiers in Plant Science, **14**.

Panse VG and Sukhatme PV (1985) Statistical methods for Agricultural Workers. ICAR, New Delhi, India, 359 p.

Rao PG, Behera TK, Munshi AD and Jat GS (2022) Genetic inheritance of fruit traits and seed coat colour in bitter gourd (*Momordica charantia*). *Indian J. Agric. Sci.* **92(3)**: 357–361.

Rathod V, Behera TK, Gaikwad AB and Hussain Z (2019) Genetic analysis and tagging of gene controlling fruit tubercles and fruit ridgeness pattern in bitter gourd using SSR markers. *Indian J. Genet. Plant. Breed.* **79(04)**: 749-755.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.04

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Agro-morphological Characterization and Digitization of Job's Tears (*Coix lacryma-jobi* L.) Germplasm: A Minor Cereal Crop of Northeastern India

Laxmisha KM[1], Kailash C. Bhatt[1*], Mamta Arya[2], Rakesh Bhardwaj[1], Dinesh P. Semwal[1] and Veena Gupta[1]

Abstract

Thirty-seven Job's tears accessions of India's northeastern hill region (NEH) were characterized for nine quantitative and eight qualitative traits. Prominent furrow and elliptic shape of grain in soft-shelled types are supposed to be the most important trait that indicates the domestication trend of the crop in NEH region. Principal component analysis showed that agro-morphological traits, namely time for first heading, days to 80% maturity, seed yield/plant (g), number of culms, and length of culm (cm) have significantly contributed to the diversity. Correlation analysis has shown significant correlations between different yield-contributing traits. Diversity and potential traits in each cluster were also identified through cluster analysis. Grid mapping was done to identify the areas for collection of trait-specific germplasm like higher number of culms, length of culm and hundred seed weight. The germplasm accessions IC0332644, IC0416831 and IC0540173 were identified as promising genotypes.

Keywords: Diversity analysis, Digitization, Job's tears, Northeastern India.

[1]ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India.

[2]ICAR-NBPGR, Regional Station Bhowali, Uttarakhand, India.

***Author for correspondence:**

kailash.bhatt@icar.gov.in

Received: 15/12/2022 **Revised:** 09/02/2023

Accepted: 10/02/2023

How to cite this article: Laxmisha, K.M., Bhatt, K.C., Arya, M., Bhardwaj, R., Semwal, D.P., Gupta, V. (2023). Agro-morphological Characterization and Digitization of Job's Tears (*Coix lacryma-jobi* L.) Germplasm: A Minor Cereal Crop of Northeastern India. Indian J. Plant Genetic Resources. 36(3), 362-370. **DOI:** 10.61949/0976-1926.2023.v36i03.04

Introduction

Coix lacryma-jobi L. (2n = 4X = 20) is commonly known as Job's tears as the shape of grain looks like a drop of tear. It is an important potential minor cereal crop that possesses various economic traits. Job's tear is an annual/perennial, monoecious taxa of the Poaceae family in tribe Maydeae but differs from other taxa in terms of fruit and inflorescence characters. Its fruit is a modified bract or hardened floral leaf, also known as a cupule, pseudocarp, or spatheole. Eastern India and Myanmar are considered as the centers of origin of Job's tears (Venkateshwarlu and Changanti, 1973). Its wild (hard-shelled) forms are distributed throughout India (except in extremely hot or dry areas), while the cultivated (soft-shelled) forms are confined to the northeastern hill region (NEH) of India and also reported to be cultivated in many south Asian countries (Jain and Banerjee, 1974; Arora, 1977). Job's tear was a more popular crop than maize as it was grown extensively in the past by aboriginals of Mongolian origin (Burkill, 1953).

Soft-shelled type of Job's tears is consumed in different forms. De-hulled matured seeds are boiled and eaten with cooked rice; flour is blended with water and taken as a cooling drink; grains are used by Garo, Naga and Karbi tribes for brewing a local liquor (Burkill, 1953) and in some places as poultry and animal feeds (Gupta *et al.*,1992). Hard-shelled types are used as ornament (Jiang *et al.*,2008); green leaves as fodder, and dried plants as thatching

material (Jain and Banerjee, 1974). Therapeutically, grains are considered useful for kidney and gall bladder stones, menstrual disorders, catarrhal infection, and roots decoction is used as a vermifuge (Jimo *et al.,*2001). Its kernel is considered a richer source of protein and carbohydrates than rice, wheat, and maize and has lower amount of phytosterols than maize (Moreau *et al.,* 2001). Although Job's tears possess high economic potential, very scanty efforts are done for its genetic improvement (Ma *et al.,*2006). Analysis of genotypic relationships is an important component for varietal development in crop plants, as it provides information on the extent of genetic diversity that serves as a platform for the stratified sampling of the breeding population. The present study aimed at agro-morphological characterization of Job's tears germplasm to know the extent of genetic diversity for utilizing it in crop improvement.

Materials and Methods

Plant material

A total of 37 selected germplasm lines of Job's tears, including three checks (IC0089385, IC0089389, and IC0089390) assembled from different states of Northeastern India (Table 1) were used in the present study. These germplasm accessions were grown in the experimental field located at the Indian Council of Agricultural Research-National Bureau of Plant Genetic Resources, Regional Station, Bhowali, Nainital (Uttarakhand) (29° 24′ 28.7′′N; 79° 30′47.2′′E; 1480 m asl). Following appropriate statistical design, all the selected accessions were sown along with checks in augmented block design with a 45 cm distance between row to row and 15 cm distance between plant and plant.

Data recording

Five plants were randomly selected from each row for recording data, and observations were taken on 17 agro-morphological traits (eight qualitative and nine quantitative) using minimal descriptors and descriptor states developed by UPOV (2012). Qualitative traits were recorded, which include seedling anthocyanin pigmentation, plant range of grain distribution, tillering habit, grain shape, the main color of grain, hardness of grain, furrow on grain, and glossiness of grain. Similarly, quantitative traits were recorded for the number of culms, culm diameter (cm), culm length (cm), length and width of leaf blade (cm), days to first heading, days to 80% maturity, hundred-grain weight (g) and grain yield/plant (g).

Statistical analysis

Statistical analysis was done using ANOVA while the mean was compared with Tukey's honest significance test, which helped determine the accessions significantly different from each other. Descriptive statistics were computed through 'R' statistic software version 3.6.2 (R Core Team, 2019) for each accession, and the mean, standard error, minimum, maximum, standard deviation, and coefficient of variation was calculated for each quantitative trait (Table 2). Principal component analysis (PCA) was calculated using the 'factoextra' package based on the data recorded on quantitative traits. Similarly, correlation analysis was done using the 'corrplot' package. Hierarchical clustering of the 37 accessions was based on the agro-morphological traits using Euclidean distances and a dendrogram was created using the 'cluster package' in R.

Table 1: Details of 37 accessions of Job's tears used in the study

Accessions	*No.*	*State*	*Status*
IC0089381, IC0089382, IC0089383, IC0089384, IC0089385, IC0089389, IC0089390, IC0089391, IC0089392, IC0089393, IC0089394	11	Meghalaya	Landrace
IC0334314, IC0334317, IC0334345, IC0374506, IC0416824, IC0416829, IC0416831, IC0416884, IC0416897, IC0332621, IC0332644	11	Arunachal Pradesh	Landrace
KCB/PKM/40	1	Arunachal Pradesh	Wild
IC0524599, IC0524631, IC0540173, IC0540222, IC0540266, IC0591727, IC0591729, IC0591730, IC0591732	9	Nagaland	Landrace
IC0330396, IC0330440, IC0330448	3	Manipur	Landrace
IC0278074, IC0278158	2	Mizoram	Landrace

Grid mapping

Geographical information system (GIS) based grid mapping technique was used to know the diversity-rich areas and occurrence of trait-specific germplasm of Job's tears. Geo-coordinates of collection sites and agro-morphological traits data of the 37 accessions of Job's tears were used for grid mapping. The simple-circular neighborhood technique of DIVA-GIS software version 7.3 was used to carry out grid analysis (Semwal *et al.,*2012; Hijmans *et al.,* 2001). Based on the diversity and area, a grid of 10 x 10 cells (111 x 111 km) size was used.

Results

Job's tears germplasm was characterized to understand the variability using standard descriptors. In the present study, striking variations were recorded for grain distribution, tillering habit, grain shape, main color of grain, grain hardness, furrow, and glossiness (Table 2). It was observed that most of the elliptically shaped grains had soft seed coats. Various categories were observed for grain color-brown (21), white (6), dark brown (6), black (2), and grey

Table 2: Details of qualitative characters of Job's tears

Acc no	*SAP*	*PRGD*	*TH*	*GS*	*GMC*	*GH*	*GF*	*GG*
IC0416831	present	narrow	upright	circular	brown	medium	absent	weak
IC0591732	present	narrow	upright	elliptic	dark brown	soft	present	weak
IC0540222	present	narrow	upright	circular	brown	medium	present	weak
IC0416884	present	narrow	upright	circular	black	soft	present	strong
IC0524599	present	narrow	upright	ovate	brown	medium	present	medium
IC0540173	present	narrow	upright	circular	black	hard	absent	strong
IC0089393	present	narrow	upright	ovate	brown	medium	present	medium
IC0332644	present	medium	upright	ovate	brown	medium	absent	strong
IC0089384	present	narrow	upright	ovate	grey	medium	present	medium
IC0334345	present	medium	upright	circular	white	hard	absent	strong
IC0089392	present	narrow	upright	circular	dark brown	hard	present	medium
IC0089391	present	narrow	upright	ovate	white	medium	present	medium
IC0089394	present	narrow	Semi-upright	ovate	brown	medium	present	weak
IC0334317	present	narrow	upright	ovate	brown	soft	absent	weak
IC0591727	present	narrow	upright	elliptic	brown	soft	present	medium
IC0330440	present	narrow	upright	circular	brown	medium	present	strong
IC0540266	present	narrow	upright	circular	brown	soft	present	medium
IC0416829	present	narrow	upright	circular	brown	soft	present	strong
IC0416897	present	narrow	upright	circular	white	medium	absent	strong
IC0524631	present	narrow	upright	circular	brown	medium	present	medium
IC0330396	present	narrow	upright	circular	brown	medium	present	strong
IC0591730	present	narrow	upright	elliptic	dark brown	soft	present	weak
IC0332621	present	narrow	upright	ovate	brown	medium	present	strong
IC0334314	present	narrow	upright	ovate	white	soft	absent	weak
IC0089382	present	narrow	upright	ovate	brown	soft	present	weak
IC0591729	present	narrow	upright	elliptic	white	hard	absent	medium
IC0089381	present	narrow	upright	elliptic	brown	Medium	present	medium
IC0330448	present	narrow	upright	circular	white	medium	present	weak
IC0089383	present	narrow	upright	ovate	dark brown	medium	present	weak
IC0089385	present	narrow	upright	ovate	brown	medium	present	medium
IC0089389	present	narrow	upright	ovate	brown	medium	present	medium
IC0089390	present	narrow	upright	circular	brown	medium	present	medium
IC0416824	present	narrow	upright	ovate	dark brown	medium	present	weak
IC0278074	present	narrow	upright	elliptic	dark brown	soft	present	medium
KCB/PKM/40	present	broad	spreading	ovate	grey	hard	absent	strong
IC0278158	present	narrow	upright	elliptic	brown	soft	present	weak
IC0374506	present	narrow	upright	ovate	brown	medium	present	medium

(Seedling anthocyanin pigmentation – SAP; plant range of grain distribution – PRGD; tillering habit – TH; grain shape – GS; grain main colour – GMC; grain hardness – GH; grain furrow – GF; grain glossiness – GG)

(2); grain hardness-soft (11), medium (21), and hard-shelled types (5); furrow on grain surface-absent (10) and present (27); and glossiness- little (12), medium (15) and high (10). The presence of traits like soft-shell, furrow on grain surface and an elliptic shape of grain indicate the trend of domestication of the crop in NEH region.

Selected agro-morphological characters were analyzed using descriptive statistics, which has shown the tremendous variation in the accessions of Job's tears (Table 3). Of these, KCB/PKM/40 (5.0), IC0524599 (2.50) and IC0332644 (2.50) were identified as superior accessions for culm numbers; IC0278084 (14.17 cm), IC0374506 (14.10 cm) and IC0524599

Table 3: Descriptive statistics values for selective morphological parameters of Job's tears

Parameters	*Mean ± SE*	*Minimum*	*Maximum*	*SD*	*CV %*
NOC	1.66 ± 0.078	1	5.0	0.70	42.31
CD	12.49 ± 0.209	9.45	14.17	1.19	9.51
LC	189.12 ± 6.215	49	298	54.00	28.55
LLB	44.85 ± 1.691	26.04	66.9	9.14	20.39
WLB	4.23 ± 0.138	2.56	7.06	0.77	18.12
TFH	130.49 ± 0.795	116	174	8.51	6.52
D80%M	172.51 ± 0.669	167	218	8.36	4.85
100 SW	7.08 ± 0.476	2.54	16.8	2.47	34.92
SYD	2.25 ± 0.526	0.12	11.69	2.79	124.13

OC – Number of culms, CD- Culm diameter (cm), LC- Length of culm (cm), LLB- Length of leaf blade (cm), WLB- Width of leaf blade, TFH – Time for first heading (days), D80%M – Days to 80% maturity (days), 100 SW – 100 seed weight (grams), SYD – Seed yield per plant (grams)].

(14.04 cm) for culm diameter; IC0540173 (298 cm), IC0334345 (290 cm) and IC0416831 (273 cm) for culm length; KCB/PKM/40 (49.0 cm), IC0278158 (64.0 cm) and IC0374506 (75.0 cm) for dwarf plant types; IC0416829 (66.90 cm), IC0089382 (64.08 cm) and IC008 9383 (57.34 cm) for leaf blade length (LLB); IC0540266 (7.06 cm), IC0089382 (5.94 cm) and IC0416829 (5.58 cm) for leaf blade width (LWB); IC0332644 (116 days), IC0332621 (120 days) and IC0416824 (120 days) for time for first heading; IC0332621 (167 days), IC0334314 (167 days) and IC0332644 (168 days) for days to 80% maturity; and IC0540173 (16.80 g), IC0330440 (11.02 g) and IC0332644 (10.47 g) for 100 seed weight. High variation in seed yield per plant, ranged from 0.12 to 11.69 g with an average of 2.25 g per plant was observed, accordingly IC0332644 (11.69 g), IC0416831 (11.39 g) and IC0089383 (8.19 g) were found as high yielders.

Analysis of variance (ANOVA)

The result of the analysis of variance has been presented in Table 4. The mean square was calculated for all the nine characters studied and significant differences ($p < 0.05$) were observed among the treatments and accessions for LC, WLB, TFH, D80%M, and SYD traits. Significant variation was also observed between the blocks for LC and WLB. Similarly, treatment vs. checks also showed significant differences for D80%M and SYD traits. No significant differences were observed among the checks for any of the parameters.

Correlation analysis

The correlation between nine different agro-morphological characters of the selected Job's tears accessions has been depicted in Figure 1. It has been observed that D80%M and TFH (0.83) traits are significantly positively correlated; NOC also has a significant positive correlation with D80%M (0.70) and TFH (0.63); and LLB with WLB (0.45). NOC has a significantly negative correlation with WLB (-0.31) and LC (-0.23), and LC has a significantly negative correlation with TFH (-0.59) and D80%M (-0.40). Similarly, WLB negatively correlates with D80%M (-0.46) and TFH (-0.36). The rest of the traits are non-significantly (positive/negative) correlated with their peers.

Principal Component Analysis (PCA)

Principal component analysis was calculated using nine quantitative traits of 37 accessions of Job's tears to depict

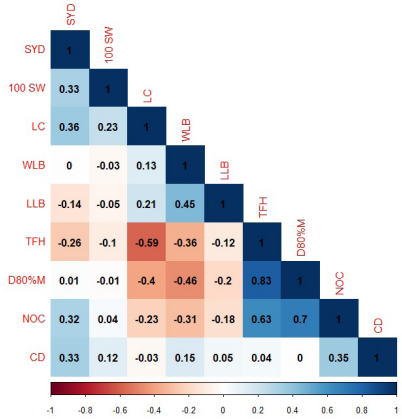


Figure 1: Correlation diagram for 9 morphological parameters of 37 accessions of Job's tears (p = 0.05)

Table 4: Analysis of variance for morphological traits of selected Job's tears accessions (*, Significant at p = 0.05)

Source (df)	*Block (3)*	*Treatment (36)*	*Treatment: Test (33)*	*Treatment: Check (2)*	*Treatment: Test vs. check (1)*	*Error (6)*
NOC	0.13	0.50	0.54	0.01	0.08	0.16
CD (cm)	0.24	1.32	1.42	0.30	0.07	1.09
LC (cm)	2960.5*	2572.8*	2755.2*	337.6	898.8	556.8
LLB (cm)	309.7	104.1	104.3	0.79	293.3	83.43
WLB (cm)	1.28*	0.66*	0.69*	0.22	0.46	0.11
DFH	19.19	67.37*	72.59*	5.25	18.37	6.02
D 80% M	0.44	66.26*	70.51*	6.33	49.79*	3.77
100 SW (g)	1.31	6.79	7.10	5.80	0.03	3.42
SYD (g)	0.43	7.75*	8.32*	0.37	8.14*	0.71

[NOC – Number of culms, CD- Culm diameter (cm), LC- Length of culm (cm), LLB- Length of leaf blade (cm), WLB- Width of leaf blade, TFH – Time for first heading (days), D80%M – Days to 80% maturity (days), 100 SW – 100 seed weight (grams), SYD – Seed yield per plant (grams)].

Table 5: Eigenvalues, the proportion of variability, and agro-morphological traits that contributed to the first three principal components (PCs) of the plant

Components	*PC1*	*PC2*	*PC3*
NOC	-0.435	0.324	-0.209
CD	-0.051	0.412	-0.501
LC	0.348	0.318	0.175
LLB	0.215	-0.113	-0.568
WLB	0.332	-0.041	-0.533
TFH	-0.514	-0.125	-0.182
D80%M	-0.512	0.047	-0.032
100 SW	0.053	0.430	0.177
SYD	0.041	0.638	0.055
Eigen values	3.075	1.854	1.318
% of Variance	34.168	20.599	14.649
Cumulative%	34.168	54.7667	69.415

[NOC – Number of culms, CD- Culm diameter (cm), LC- Length of culm (cm), LLB- Length of leaf blade (cm), WLB- Width of leaf blade, TFH – Time for first heading (days), D80%M – Days to 80% maturity (days), 100 SW – 100 seed weight (grams), SYD – Seed yield per plant (grams)].

the overall diversity in the crop. The PCA (Table 5) indicates that the majority of the required information can be drawn from the first three PCs having an Eigenvalue above 1, which have cumulatively contributed about 69.42% of the total variation. The traits TFH, D80%M and NOC have significantly contributed toPC1. This indicated that PC1 with an Eigenvalue of 3.08 and 34.17% variance of the total variation, has contributed mainly to ontogeny and vegetative growth of the crop. In PC2, SYD and 100SW traits have contributed more to yield parameters, with an Eigenvalue of 1.85 and 20.60% variance of total variation observed in the studied genotypes. The traits like LLB, WLB, and CD have contributed more in PC3 for the plant's vegetative growth with an Eigenvalue of 1.32 and 14.65% variance. In the bi-factorial plane, the contribution and correlation of the agro-morphological traits are indicated inside the circle. Morphological characters TFH, D80%M, SYD, NOC, and LC contributed more to the variability (Figure 2).

Cluster analysis

The agro-morphological traits of Job's tears accessions used in the present study were grouped into six different clusters using the hierarchical Euclidean cluster analysis method (Figure 3), while the mean of each cluster is presented in Table 6. Cluster I consists of only one accession KCB/PKM/40 (wild), represented from Arunachal Pradesh, showing above the mean cluster value for NOC (5.0), TFH (174 days), and D80%M (218 days) traits. Cluster II, which consists of three accessions (IC0332644, IC0416831, and IC0540173) representing Arunachal Pradesh (2) and Nagaland (1) have shown higher mean cluster value for NOC (2.15), CD (13.45 cm), LC (271.33 cm), TFH (121 days), 100SW (11.08 g) and SYD (9.76 g) traits. Cluster III, which consists of 12 accessions (IC0089382, IC0089383, IC0089384, IC0089385, IC0089389, IC0089390, IC0089393, IC0334345, IC0416829, IC0524599, IC0540222 and IC0540266) representing Meghalaya (7) and Arunachal Pradesh (5) have shown above mean cluster value for LC (210.48), LLB (53.92 cm) and WLB (4.67 cm) traits. Cluster IV, which consists of only one accession

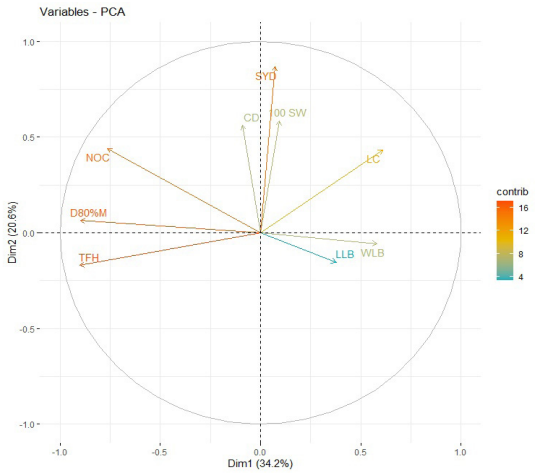


Figure 2: Principal component and variable correlation plot for 9 agro-morphological traits

Table 6: Cluster means for various agro-morphological traits of Job's tears

Parameters	*Cluster I*	*Cluster II*	*Cluster III*	*Cluster IV*	*Cluster V*	*Cluster VI*
NOC	5.00	2.15	1.73	1.00	1.15	1.59
CD	12.95	13.45	12.99	9.45	11.42	12.67
LC	49.00	271.33	210.48	154.00	187.55	162.88
LLB	33.00	40.65	53.92	51.86	45.07	37.10
WLB	2.56	4.02	4.67	3.60	4.33	3.97
TFH	174.00	121.00	130.02	133.00	128.75	130.67
D80% M	218.00	171.00	171.08	182.00	169.63	171.67
100 SW	6.07	11.08	6.75	10.41	5.35	7.38
SYD	1.09	9.76	2.38	0.80	0.85	1.40

[NOC – Number of culms, CD- Culm diameter (cm), LC- Length of culm (cm), LLB- Length of leaf blade (cm), WLB- Width of leaf blade, TFH – Time for first heading (days), D80%M – Days to 80% maturity (days), 100 SW – 100 seed weight (grams), SYD – Seed yield per plant (grams)].

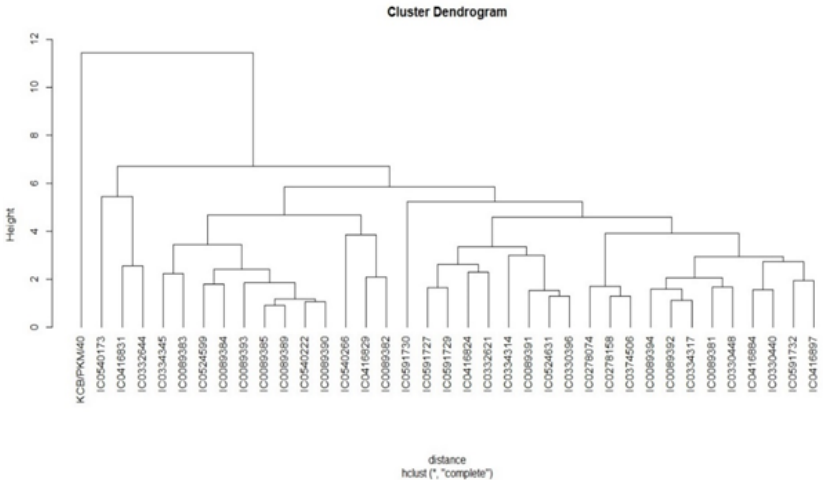


Figure 3: Cluster diagram for 9 morphological parameters of 37 accessions of Job's tears

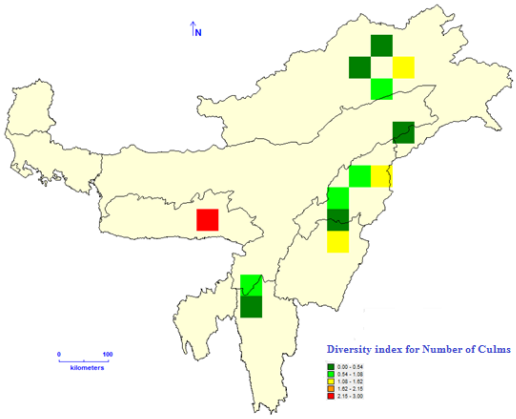


Figure 4: Grid map showing trait-specific germplasm for number of culms from different regions of north-eastern India

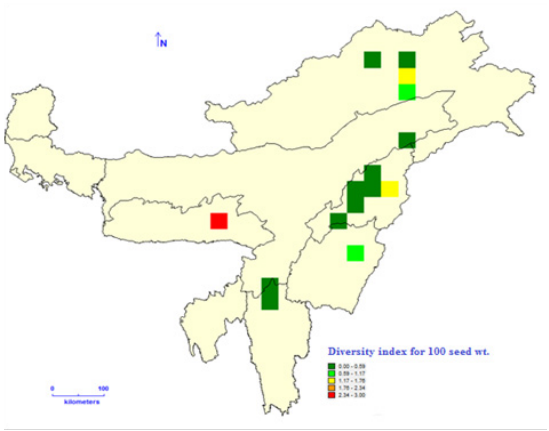


Figure 6: Grid map showing trait-specific germplasm for 100 seed weight from different regions of north-eastern India

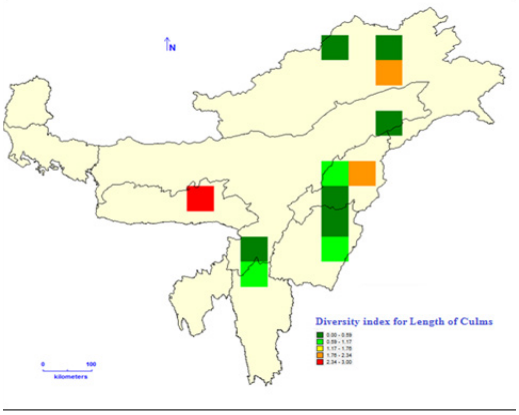


Figure 5: Grid map showing trait-specific germplasm for the length of culms from different regions of north-eastern India

(IC0591730) representing Nagaland, has shown an above-average cluster mean for LLB (51.86 cm) and 100SW (10.41 g) traits. Cluster V consists of eight accessions (IC0089391, IC0330396, IC0332621, IC0334314, IC0416824, IC0524631, IC0591727 and IC0591729), representing Arunachal Pradesh (3), Nagaland (3), Meghalaya (1) and Manipur (1). Cluster VI consists of twelve accessions (IC0089381, IC0089392, IC0089394, IC0278074, IC0278158, IC0330440, IC0330448, IC0334317, IC0374506, IC0416884, IC0416897 and IC0591732), representing Arunachal Pradesh (4), Meghalaya (3), Manipur (2), Mizoram (2) and Nagaland (1) states. Both clusters V and VI showed below-average cluster mean values for most of the traits.

Digitization of trait-specific germplasm

The promising accessions identified with superior characteristics for the length of culm, number of culms, 100 seed weight and seed hardness were mapped using grid mapping to identify the areas for the collection of trait-specific germplasm from the northeastern hill regions of India. The regions represented by red color depict high diversity for the number of culms (Figure 4), length of culm (Figure 5), 100 seed weight (Figure 6), and the regions represented by dark green color depict high diversity soft-shelled seed types (Figure 7). Grid map also showed that Job's tears accessions identified as promising for traits like

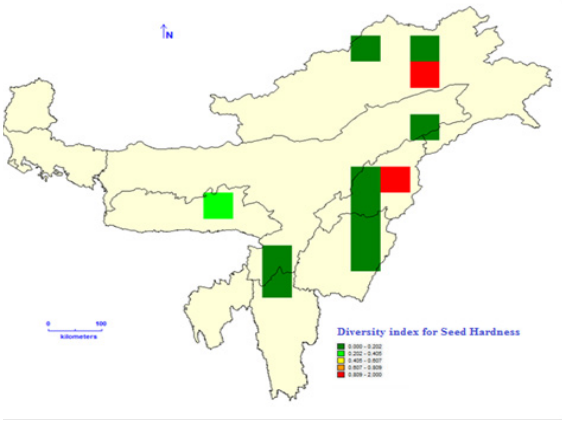


Figure 7: Grid map showing trait-specific germplasm for seed hardness from different regions of north-eastern India

the number of culms, length of culm, and 100 seed weight can be collected from East and West Khasi Hills districts of Meghalaya (Figure 4, 5 & 6); germplasm for soft-shelled type can be collected from Upper Siang and Dibang Valley districts of Arunachal Pradesh; Kohima and Phek districts of Nagaland; and West Imphal, Ukhrul and Kamjong districts of Manipur (Figure 7).

Discussion

Studying agro-morphological characters is the first step in the characterization of the germplasm of any crop (Smith *et al.,* 1991). Proper characterization of germplasm helps in understanding the unambiguous segregation between accessions, distinguishing redundancies and checking genetic change during maintenance. Hence, the characterization of genetic diversity is essential to identify suitable parents for breeding programs (Karihaloo, 2004). Job's tears is considered as a distant relative of maize (Celarier, 1957). Furthermore, unlike its occidental relative maize, Job's tears have not lost the wild types from which the cultivated types arose so it is still possible to study the reasons for its transition from wild state to domesticated state and the causal factors. The soft-shelled forms presently grown in the NEH region result from conscious folk domestication and must have been selected for easy hulling and desired kernel type. Taxonomically, *C. lacryma-jobi* var. *lacryma-jobi* is considered as wild and *C. lacryma-jobi* var. *ma-yuen,* as domesticated form (Pradheep *et al.,* 2014). The involucre of the wild type is hard and non-striated, whereas it is soft and striated in cultivated form (Jain and Banerjee, 1974). Similar results were obtained in the present study also (Table 2), hence can be concluded that the presence of prominent striation and seed softness are the probable traits of domestication.

The present study has revealed tremendous variation in traits like SYD, 100 SW, NOC, and LC (Table 3), which provides enough opportunities for utilizing them in the improvement of Job's tear. Plant height is positively correlated with seed yield per plant (Azarpour *et al.,* 2012), but sometimes dwarf plants also indirectly add to the harvest index towards the yield per plant (Avila *et al.,* 2007). In some accessions, variation in the length of the culm was recorded from 49 cm (KCB/PKM/40) to 298 cm (IC0540173), which was different than the results obtained by Kumar *et al.,* (2017). Culm length and its diameter are hardly affected by planting density (Nakano *et al.,* 2013), because higher planting density lowers the stem growth, which in turn affects the yield. According to a study by Kumar *et al.*,(2017), D 80% M is around 149 days, while in the present study 167 days were recorded in IC0332621 and IC0334314, which are found to be late in maturity. It indicates that the environment plays a significant role in the period of crop maturity. The trait 100 seed weight determines yield per plant, whereas seed yield per plant is determined by a combination of factors like 100-grain weight, grain number, and effective panicles (Yao *et al.,* 2013). The present study identified IC0332644 (11.69 g) and IC0416831 (11.39 g) as superior accessions for the trait SYD.

The analysis of variance provides a basis for the test of significance (Dospekhov, 1984). Among the accessions studied, a significant difference was observed for the traits: LC, WLB, TFH, D80%M, and SYD. This indicates that enough heritability is available for conducting varietal development trials of the crop. A non-significant difference was also observed among the checks, which indicates that the performance of all the checks was similar (Nurmala *et al.,* 2017). The traits like TFH and D80%M are significantly positively correlated; it shows that early flowering accessions can be considered as early maturing types while Job's tears is generally a late maturing crop. NOC has a significant positive correlation with D80%M and TFH, which shows that if NOC increases then much time will be required for the maturity of the crop. LLB is significantly positively correlated with WLB; it indicates that with the increase of LLB, WLB will be automatically increased. An increase in leaf area index indicates an increase in the seed weight (Shakoor *et al.,* 2007). NOC has a significant negative correlation with LC and WLB, which means as the number of tillers increases, a decrease in the height of the plant and size of the leaf can be seen, which shows that the higher the number of tillers, the partition of energy between vegetative and reproductive growth stages may be difficult, which leads to reduced growth. This was mainly observed in wild accession (KCB/PKM/40) of the Job's tears. Similarly, a significant negative correlation of LC and WLB was also observed for TFH and D80%M, which means dwarfness of the plant leads to late maturity. Only wild accession (KCB/PKM/40) has shown such a tendency, which may be clearly due to genetic factors. Hasan *et al.,* (2011) has also observed similar results in the case of cultivated and wild rice accessions.

Principal component analysis (PCA) was carried out to know about the impact of individual traits on the overall

variability. It helps in trait-specific breeding by recognizing the important economical traits and is an effective method of crop evaluation (Wang *et al.,* 2013). In the present study using the PCA method, nine morphological traits were reduced to three main components with a cumulative variance of 69.42%. Traits like TFH, D80%M, and NOC contributed much in the generation of variability (Figure 2) and are categorized as PC1 (Table 5). Being an important contributor of the variability, SYD (Figure 2) and 100SW were categorized in PC2 (Table 5). LC, a major contributor to overall diversity (Figure 2) didn't load much in any single PC (Table 5). Hence, nine agro-morphological characters can be reduced to five comprehensive traits to delineate the original data as well as to reveal the relationship among the parameters. Such traits are helpful in parental selection in a breeding program (Li *et al.,* 2010).

Hierarchical clustering helps to assess the degree of relationship between the genotypes (Ward, 1963). In the present study, 37 accessions were grouped into 6 clusters (Figure 3). The clustering pattern did not show any relationship between geographical distribution and genotypic diversity as accessions of different geographical origins formed one cluster. Similar result was observed by Forsberg *et al.,*(2015). This shows that location differences do not influence genetic variations, while germplasm from the same locality can be genetically different (Li *et al.,* 2010). In cluster I, KCB/PKM/40 has the highest number of tillers but is late maturing accession. Cluster II contains accessions IC0332644, IC0416831, and IC0540173, which have high mean cluster values for most of the traits. Hence, these accessions are considered superior and can be selected as parents for further crop improvement programs. Cluster III has accessions with high vegetative growth traits like LC, LLB, and WLB, which can be used in forage breeding programs.

Grid maps are generated to recognize the areas with species richness or niches where Job's tears is predominantly grown (Semwal *et al.,* 2012). Using grid maps, areas for future exploration and collection of trait-specific germplasm have been identified. Germplasm of Job's tears having traits like high number of culms, high culm length, and high 100 seed weight (Figures 4-6) can be collected from East and West Khasi Hills of Meghalaya, and soft-shelled type (Figure 7) from Upper Siang and Dibang Valley districts of Arunachal Pradesh; Kohima and Phek districts of Nagaland; and West Imphal, Ukhrul and Kamjong districts of Manipur.

Conclusion

Agro-morphological characterization of Job's tears showed high variability among the selected genotypes. Data analysis of characterized genotypes has indicated that the accessions IC0332644, IC0416831, and IC0540173 were superior among the studied germplasm and, hence can be utilized in the improvement of Job's tears. Principal component analysis has shown that five comprehensive traits can delineate the original data and indicate the relationship among the parameters. The clustering pattern did not show any relationship between geographical distribution and genotypic diversity. The present study indicates that there is a need to enrich the National Genebank by undertaking more explorations in the NEH region to collect germplasm, as many remote localities are still unexplored.

Moreover, fine grid surveys are also required to undertakefor the collection of trait-specific germplasm, considering the promising accessions identified. The inhabitants also need to be made aware of the importance of Job's tears as it is a nutritionally rich minor cereal. Hence, on-farm crop conservation is required to face the challenges of climate change.

Acknowledgments

The authors are thankful to the Director, ICAR-NBPGR, and ICAR-IARI, New Delhi for providing support and guidance in conducting the experiment. Mr. Ashvin Katral, ICAR-IARI, is also acknowledged for extending help in statistical analysis.

References

Arora, R.K. (1977). Job's tears (*Coix lacryma- jobi*) - a minor food and fodder crop of North-eastern India. *Econ. Bot*., **31**: 358-366.

Avila, C.M., Atienza, S.G., Moreno, M.T. & Torres A.M. (2007). Development of a new diagnostic marker for growth habit selection in faba bean (*Vicia faba* L.) breeding. *Theor. Appl. Genet*., **115**: 1075-1082.

Azarpour, E., Bidarigh, S., Moraditochaee, M. Danesh, R.K., Bozorgi, H.R. & Bakian, M. (2012). Path coefficient analysis of seed yield and its components in faba bean (*Vicia faba* L.) under nitrogen and zinc fertilizer management. *Int. J. Agric. Sci*., **4**: 1559-1561.

Burkill, I.H. (1953). The Hooker Lecture: Habits of the man and origin of the cultivated plants of the old world. In Proceedings of the Linnean Society of London, **164(1)**:12-42.

Celarier, R.P. (1957). Cytotaxonomy of the Andropogoneae II Subtribes Ischaeminae, Rottboelliinae, and the Maydeae. Cytologia, **22**: 160-183.

Dospekhov, B.A. (1984). Field experimentation: statistical procedures. Mir Publishers, Moscow.

Forsberg NE, J Russell, M Macaulay, MW Leino & J Hagenblad (2015) Farmers without borders-genetic structuring in century-old barley (Hordeum vulgare). Heredity, **114**: 195-206.

Gupta, J.J., Gupta, H.K. & Yadav, B.P.S. (1992). Decorticated Job's tears (Coix lacryma- jobi) grain as poultry feed. *Indian. J. Anim. Sci.*, **62**: 699-700.

Hasan, M.J., Kulsum, M.U., Akter, A., Masuduzzaman, A.S. & Ramesha, M.S. (2011). Genetic variability and character association for agronomic traits in hybrid rice (*Oryza sativa* L.). Bangladesh. *J. Plant Breed. Genet*., **24**: 45-51.

Hijmans, R.J., Guarino, L. Cruz, M. & Rojas, E. (2001). Computer tools for spatial analysis of plant genetic resources data: 1. DIVA-GIS. Plant Genetic Resources Newsletter, pp15-19.

Jain, S.K. & Banerjee, D.K. (1974). Preliminary observations on the ethnobotany of the genus *Coix*. *Econ. Bot*. 38-42.

Jiang, H.E., Wang, B. Li, X. Lü, E.G. & Li, C.S. (2008). A consideration

of the involucre remains of Coix lacryma-jobi L. (Poaceae) in the Sampula Cemetery (2000 years BP), *Xinjiang, China. J. Archaeol. Sci.*, **35**: 1311-1316.

Jimo, P.V., Singh, R.A., Achuni, T.I. & Singh, P.I. (2001). Job's tears: an under-exploited and medicinal crop of North-East Hill region. *Nat. Agric. Sci. Cong*., pp: 49.

Karihaloo, J.L. (2004). Advances in molecular characterization of plant genetic resources and assessment of diversity. Plant Genetic Resources Management, Narosa Publishing House, New Delhi, pp: 266-274.

Kumar, R., Yhokha, K., Rajesha, G. & Deka, B.C. (2017). Performance of Job's tears lines (Coix lacryma-jobi) under foothill condition of Nagaland. *Environment & Ecology*, **35**: 440-444.

Li, S., Li, X., Wang, S. & Zhang, Z. (2010). Clustering and principal component analysis of introduced black pericarp rice germplasm based on agronomic traits. Southwest China. *J. Agric. Sci*., **23**:11-15.

Ma, K.H., Kim, K.H., Dixit, A., Yu, J.W., Chung, J.W., Lee, J.H., Cho, E.G., Kim, T.S. & Park, Y.J. (2006). Newly developed polymorphic microsatellite markers in Job's tears (*Coix lacryma-jobi* L.). *Mol. Ecol. Notes*., **6**: 689-691.

Moreau, R.A., Singh, V. & Hicks, K.B. (2001). Comparison of oil and phytosterol levels in germplasm accessions of corn, teosinte, and job's tears. *J. Agric. Food Chem*., **49**: 3793-3795.

Nakano, H., Ujihira, Y. & Ishida, K. (2003). Correlation of planting density with yield and yield components in Job's-tear plant (*Coix lacryma-jobi* L. var. frumentacea Makino). *Jpn. J. Crop Sci*., **72(1)**: 32-37.

Nurmala, T. & Wicaksono, F.Y. (2017). Growth and yield of job's tears (*Coix lacryma-jobi* L.) response to different types of Oldeman climate classification and row spacing in West Java Indonesia. J. Agron., **16**: 76-82.

Pradheep, K., Bansal, K.C. & Bhandari, D.C. (2014). Wild relatives of cultivated plants in India. Directorate of Knowledge Management in Agriculture, Indian Council of Agricultural Research, p 728.

R Core Team. (2019). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. *https://www.R-project.org/*

Semwal, D.P., Bhandari, D.C., Bhatt, K.C. & Singh, R. (2012). Diversity distribution pattern in collected Germplasm of Rapeseed-Mustard using GIS in India. *Indian J. Plant Genetic Resources*., **26**: 76-81.

Shakoor, M.S., Akbar, M. & Hussain, A. (2007). Correlation and path coefficients studies of some morphophysiological traits in maize double-crosses. *Pak. J. Agri. Sci*., **44**: 213-216.

Smith, S.E., Al-Doss, A. & Warburton, M. (1991). Morphological and agronomic variation in North African and Arabian alfalfas. *Crop Sci*., **31**: 1159-1163.

UPOV document TG/18/1. (2012). Guidelines for the conduct of tests for distinctness, uniformity, and stability (DUS).

Venkateswarlu, J. & Chaganti, R.S.K. (1973). Job's tears (*Coix lacryma-jobi* L.). ICAR Tech. Bull, p 44.

Wang, S., Zhang, S., He, J., Lu, G. & Yang, Z. (2013). The principal component analysis and cluster analysis of Coix resource characteristics. Journal of Yunnan Agricultural University, **28**: 157-162.

Ward, J.H. (1963). Hierarchical grouping to optimize an objective function. *J. Am. Stat. Assoc*., **58**: 236-244.

Yao, F., Yin, G., Mo, G., Luo, Y. and Yuan, J. (2013). *Coix lacryma-jobi* L. growth and yield response to different sowing dates in Sichuan *China. J. Sci. Applic. Biomed*., **1**: 8-14

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.05

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Screening of Wild and Cultivated *Vigna* Accessions for Resistance to Bruchid (*Callosobruchus maculatus* F.)

Ragul Subramaniyan[1,2] and Manivannan Narayana[2*]

Abstract

The bruchid beetles (*Callosobruchus maculatus)* is one of the primary causes for the loss in pulses due to the seed damages during storage. The wild relatives of crop species are essential sources of new genes for crop improvement. A set of 40 wild *Vigna* accessions and cultivated *Vigna* species were screened for C. *maculatus* resistance. Based on the results, the accessions *viz*., *V. radiata* var. *sublobata*, *V. trilobata* and *V. umbellata* were found resistant as it recorded less adult emergence, lesser seed damage, low index of susceptibility and less seed weight loss among bruchid resistance traits. These traits are more influential principal components and have a high correlation. The identified accessions can be involved in the resistance breeding program as a donor to introgress resistance against bruchid beetles towards developing superior cultivars. One of the identified key traits, *viz.,*seed weight loss or seed damage or index of susceptibility, should be given more importance while framing the breeding program for reliable and stable bruchid resistance among the cultivar.

Keywords: Bruchid, *Callosobruchus maculatus*, Resistance, *Vigna* species, Wild genetic sources.

[1]National Pulses Research Centre, Tamil Nadu Agricultural University, Vamban, Tamil Nadu, India.

[2]Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

***Author for correspondence:**

nm68@tnau.ac.in

Received: 20/12/2022 **Revised:** 11/04/2023

Accepted: 03/10/2023

How to cite this article: Subramaniyan, R., Narayana, M. (2023). Screening of Wild and Cultivated Vigna Accessions for Resistance to (*Callosobruchus maculatus* F). Indian J. Plant Genetic Resources. 36(3), 371-381.
DOI: 10.61949/0976-1926.2023.v36i03.05

Introduction

Pulses are an important primary source of dietary proteins and essential amino acids in human nutrition and are consumed extensively in Asian and African countries. It belongs to the *Fabaceae* family (Asif *et al*., 2013). It includes black gram (*Vigna mungo*), green gram (*V. radiata*), adzuki bean (*V. angularis*), cowpea (*V. unguiculata*) pigeon pea (*Cajanus cajan*), moth bean (*V. aconitifolia*) and rice bean (*V. umbellata*). Globally, pulse crops serve as the second most important group of crop plants next to cereals. India is a major producer, consumer and importer of pulse crops among the pulse-growing countries. India has contributed about 3.56 million tonnes of pulses production per annum from 5.44 million hectares of area with a productivity of 655 kilograms per hectare (Anonymous, 2019). Though India has achieved consistent milestones in the production of pulses over the last decades, the consumption of pulses has been increasing a lot. With ever-increasing demand for pulses across the country, the production needs to be upscaled by increasing productivity. In addition to its production shortage, the net quantum of productivity and economic value is constantly being affected by storage pests, especially the bruchid beetles (*Callosobruchus maculatus*).

Bruchid beetles (cowpea weevil), *C. maculatus* (Chrysomelidae: Bruchinae) cause loss in both quantity and quality during storage in tropics and sub-tropical areas (Duraimurugan *et al*., 2011). The infestation begins from the field; however, the devastating losses are realized only during the storage due to their continuous

perpetuation in stored lots, which might inflict grain losses ranging from 40 to 90% and sometimes 100%, if left unnoticed. Management of bruchid beetles has remained challenging over decades (Mishra *et al.,* 2017). Furthermore, these control methods are cost-ineffective, impractical, and non-sustainable in the economic, health, and environmental safety concerns (Credland, 1994). Hence, alternate and sustainable strategies are required to tackle bruchid infestations (Kashiwaba *et al.,* 2003). Host plant resistance (HPR) is a traditional yet highly effective method. It is constantly adapted to the identify the essential traits in the host plants against the insect pests (Pratap and Gupta, 2009).

In this regard, identifying bruchid resistance in wild species, traditional cultivars or landraces using proper screening approaches is inexpensive and sustainable in the long run (Began *et al.,* 1990). Hence the best option to cope with the present situation is to introgression the essential alien genes from crop wild relatives to diversify and widen the genetic base of legumes (Pratap *et al.,* 2020). Attempts towards identifying of resistant sources were undertaken in many wild legumes, especially in *Vigna* subgenus *ceratotropics*. However, the successful transfer of resistance genes to cultivated species was somehow lagging due to several issues (Nair *et al.,* 2019). The wild relatives of crop species are important sources of new genes for crop improvement. Greater availability of genetic diversity was measured at both the biochemical and DNA level in wild species than their closely related species (Harlan, 1984; Xu *et al*., 2000). Therefore, evaluating a broad range of wild species is appropriate for exploring genes unavailable in cultigens. The subgenus *ceratotropis* of the genus *Vigna* is an important taxonomic group because it includes seven cultivated species, green gram [*Vigna radiata* (L.) Wilczek], blackgram [*V. mungo* (L.) Hepper], moth bean [*V. aconitifolia* (Jacquin) Maréchal], azuki bean [*V. angularis* (Willdenow) Ohwi & Ohashi], rice bean [*V. umbellata* (Thunberg) Ohwi&Ohashi], jungli bean [*V. trilobata* (L.) Verdcourt] and *V. reflexo-pilosa* Hayata subsp. *Glabra* (Roxburgh) Tateishi (Tateishi, 1985; Lawn, 1995). Though the studies were found successful in the identification of the resistance sources from diverse genetic resources in wild *Vigna* and its derived varieties yet the attempts towards the transfer resistance into cultivated ones remained limited (Sarkar *et al.,* 2011; Seram *et al,* 2016; Subramaniyan *et al,* 2021).

Many authors attempted crosses to incorporate the bruchid resistance from wild *Vigna* accessions into cultivated ones (Tomooka *et al.,* 2000; Sun *et al.,* 2008; Souframanien *et al*., 2010; War *et al*., 2017). However, the expected outcome of high yield accompanied with resistance was not achieved due to limited resistant source. Hence, it is essential to identify more genetic resources from the wild and the relatives of *Vigna* to support the research towards the transfer of essential genes. Thus, the present study was formulated with the following objectives, i) to find new sources of resistance against *C. maculatus,* ii) to ascertain the diverse nature of 40 *Vigna* accessions, cultivars and wild *Vigna* towards the bruchid resistance (*C. maculatus*) and iii) to find the essential trait among the bruchid resistance traits for proper classification of resistant accessions.

Material and Methods

Experimental design

The present experimental study was conducted from February to April 2020. A completely randomized design was followed with three replications at the Entomology laboratory, National Pulses Research Centre (NPRC), Tamil Nadu Agricultural University, Vamban, India.

Vigna Species

Seeds of 40 *Vigna* accessions that belong to seven different *Vigna* species groups were the base material for the study. The seeds were obtained from the NPRC, TNAU, Vamban, Tamil Nadu (India) wild species garden. The wild *Vigna* species and cultivated *Vigna* species with its taxon group, accession number and collection place were summarized in Supplementary Table 1 with its qualitative traits *viz.,* seed color, seed shape, seed luster and seed size (in terms of 100 seed weight). The seed characterization is based on the guidelines for the conduct of distinctiveness, uniformity and stability evaluation published by the Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA), Government of India. Seeds of each accession were stored at -20°C for 48 hours to avoid carry-over infestation from the field.

Insect culture

Among the various species, *C. maculatus* covers the major proportion of nearly 90% in the seed lots at Vamban. Beetles were collected from the storage lots of NPRC, Vamban and multiplied on green gram seeds of VBN 4 [*Vigna radiata* (L.) Wilczek] variety at constant temperature of 29˚C and 70% relative humidity. Good aeration was provided through tiny pinholes on the sides of the container. The cowpea beetle lacks the "snout" of a true weevil. It is reddish-brown in color, with black and gray elytral markings with two black spots at center. The abdomen extends out from the elytra and has two black spots in the last segment of the body. It is sexually dimorphic. Specifically, males are easily distinguished from females. The females look larger than the males. Females are darker in color than males, while males are brown in color. The beetles were identified morphologically from the other bruchid species with two key traits such as a) the presence of less dense setae on the ventral side of the 2,3,4 abdominal segments (sternites) and b) presence of a serrate type of antenna in both males and females, respectively. Freshly emerged adults were collected from the stock culture and used for bioassay.

Table 1: Screening of wild *Vigna* accessions against *C. maculatus* infestation

S. No	Species	No. of eggs/50 seeds ± SE	Mean No. of eggs/seed ± SE	Develop-mental time (days) ± SE	Total no. of adult emergence ± SE	Mean developmental period (days) ± SE	Index of susceptibility (IS) ± SE	Seed damage (%) ± SE	Seed weight loss (%) ± SE
	V. glabrescens								
1	IC251372	185.3 ± 5.8	3.7 ± 0.1	27.7 ± 0.3	37.3 ± 1.5	36.4 ± 0.2	8.6 ± 0.1	74.7 ± 2.9	46.7 ± 2.6
	V. mungo var. *silvestris*								
2	TCR265	91.3 ± 2.9	1.8 ± 0.1	23.0 ± 0.0	45.7 ± 1.8	36.0 ± 0.5	9.2 ± 0.2	91.3 ± 3.5	65.3 ± 0.6
	V. radiata var. *sublobata* (Roxb.) Verdcourt								
3	TCR218	81.0 ± 5.0	1.6 ± 0.1	26.0 ± 0.6	28.0 ± 4.5	38.0 ± 1.9	7.6 ± 0.8	56.0 ± 9.0	35.9 ± 6.4
4	TCR188	87.0 ± 2.3	1.7 ± 0.0	55.0 ± 0.0	0.0 ± 0.0	- ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.4
5	V. trilobata	59.0 ± 1.7	1.2 ± 0.0	55.0± 0.0	0.0 ± 0.0	- ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.2 ± 1.6
	V. umbellata (Thunb.) Ohwi & H. Ohashi								
6	RBHP-109-1	239.7 ± 10.2	4.8 ± 0.2	33.3 ± 1.8	32.0 ± 2.5	44.3 ± 0.7	6.8 ± 0.1	64.0 ± 5.0	30.3 ± 5.0
7	IC528870-2-36	147.7 ± 25.9	3.0 ± 0.5	36.7 ± 1.2	21.0 ± 8.1	46.0 ± 1.3	5.3 ± 0.9	42.0 ± 16.3	24.4 ± 8.8
8	IC528870-1-63	194.0 ± 19.1	3.9 ± 0.4	41.7 ± 1.7	13.0 ± 3.6	48.6 ± 0.7	4.4 ± 0.7	26.0 ± 7.2	14.2 ± 3.1
9	IC528870-6-93	167.3 ± 8.7	3.3 ± 0.2	35.7 ± 0.3	22.7 ± 1.5	46.8 ± 0.2	5.8 ± 0.1	45.3 ± 2.9	23.5 ± 2.5
10	IC528870-2-54	131.7 ± 1.5	2.6 ± 0.0	37.7 ± 1.5	9.7 ± 0.7	47.1 ± 0.3	4.2 ± 0.2	19.3 ± 1.3	13.9 ± 3.3
11	IC528870-2-78	192.0 ± 3.2	3.8 ± 0.1	36.0 ± 0.6	21.7 ± 2.0	46.2 ± 0.4	5.8 ± 0.2	43.3 ± 4.1	26.3 ± 4.0
12	IC528870-1-11	180.3 ± 4.1	3.6 ± 0.1	33.0 ± 0.0	26.7 ± 2.2	43.8 ± 0.6	6.5 ± 0.2	53.3 ± 4.4	34.0 ± 5.9
13	IC528870-4-42	136.7 ± 10.1	2.7 ± 0.2	36.3 ± 0.9	24.7 ± 1.8	47.0 ± 0.6	5.9 ± 0.1	49.3 ± 3.5	20.3 ± 1.3
14	ICP1871-71	70.7 ± 4.9	1.4 ± 0.1	31.0 ± 0.6	15.3 ± 4.1	43.6 ± 0.5	5.3 ± 0.6	30.7 ± 8.1	10.9 ± 1.9
15	IC137171-5	104.7 ± 5.0	2.1 ± 0.1	32.3 ± 0.3	15.0 ± 0.6	45.6 ± 0.1	5.2 ± 0.1	30.0 ± 1.2	10.7 ± 0.9
16	RED-5	188.7 ± 5.8	3.8 ± 0.1	33.7 ± 0.7	29.0 ± 4.0	44.6 ± 0.5	6.5 ± 0.3	58.0 ± 8.0	41.2 ± 0.5
17	RBHP 109-2	66.0 ± 2.6	1.3 ± 0.1	39.3 ± 0.3	4.0 ± 1.7	41.8 ± 0.9	2.3 ± 1.2	8.0 ± 3.5	2.3 ± 1.1

Table 2: Screening of cultivated *Vigna* accessions against *C. maculatus* infestation

S. No	Species	No. of eggs/50 seeds ± SE	Mean no. of eggs/seed ± SE	Developmental time ± SE	Total no. of adult emergence ± SE	Mean developmental period (days) ± SE	Index of susceptibility (IS) ± SE	Seed damage (%) ± SE	Seed weight loss (%) ± SE
	Cross derivatives of *V. mungo* cv. BDR1 x *V. mungo* var. *silvestris*								
1	VBG 19-001	163.3 ± 2.4	3.3 ± 0.0	35.0 ± 0.0	41.0 ± 2.1	49.1 ± 0.3	6.6 ± 0.1	82.0 ± 4.2	47.5 ± 1.5
2	VBG 19-002	175.7 ± 4.1	3.5 ± 0.1	35.3 ± 0.3	45.7 ± 1.9	49.6 ± 0.2	6.7 ± 0.1	91.3 ± 3.7	49.3 ± 1.2
3	VBG 19-003	251.7 ± 4.6	5.0 ± 0.1	28.0 ± 1.5	47.3 ± 0.3	48.0 ± 0.1	7.0 ± 0.0	94.7 ± 0.7	52.0 ± 0.4
4	VBG 19-004	245.7 ± 7.0	4.9 ± 0.1	35.0 ± 0.0	44.7 ± 0.3	47.5 ± 0.5	7.0 ± 0.1	89.3 ± 0.7	49.3 ± 3.7
5	VBG 19-005	230.3 ± 5.5	4.6 ± 0.1	31.0 ± 0.6	49.7 ± 1.3	48.6 ± 0.5	7.0 ± 0.0	98.0 ± 2.0	46.5 ± 2.2
6	VBG 19-006	208.3 ± 1.7	4.2 ± 0.0	31.3 ± 0.7	47.7 ± 0.3	48.3 ± 0.3	6.9 ± 0.0	95.3 ± 0.7	53.4 ± 0.5
7	VBG 19-007	222.3 ± 5.4	4.4 ± 0.1	32.7 ± 0.3	37.0 ± 0.6	48.4 ± 0.4	6.5 ± 0.1	74.0 ± 1.2	49.9 ± 0.8
8	VBG 19-008	240.0 ± 4.0	4.8 ± 0.1	30.7 ± 0.7	41.0 ± 4.0	48.5 ± 0.2	6.6 ± 0.2	82.0 ± 8.0	52.4 ± 0.5
9	VBG 19-009	144.7 ± 5.5	2.9 ± 0.1	29.0 ± 0.6	48.3 ± 0.7	48.4 ± 0.2	7.0 ± 0.0	96.7 ± 1.3	53.1 ± 2.6
10	VBG 19-010	210.0 ± 5.8	4.2 ± 0.1	33.7 ± 0.3	34.0 ± 5.6	48.8 ± 0.4	6.2 ± 0.3	68.0 ± 11.1	48.8 ± 0.9
11	VBG 19-011	243.7 ± 7.3	4.9 ± 0.1	27.7 ± 0.3	39.7 ± 4.1	48.3 ± 0.3	6.6 ± 0.2	79.3 ± 8.1	50.7 ± 1.4
12	VBG 19-012	228.3 ± 10.1	4.6 ± 0.2	29.3 ± 0.7	38.0 ± 1.5	48.4 ± 0.7	6.5 ± 0.2	76.0 ± 3.1	43.7 ± 0.8
13	VBG 19-013	224.7 ± 15.6	4.5 ± 0.3	33.7 ± 0.7	40.3 ± 1.5	49.1 ± 0.2	6.5 ± 0.1	80.7 ± 2.9	48.7 ± 1.4
14	VBG 19-014	270.0 ± 9.1	5.4 ± 0.2	29.7 ± 1.2	46.0 ± 1.2	47.7 ± 0.6	7.0 ± 0.1	92.0 ± 2.3	50.8 ± 3.0
15	VBG 19-015	167.3 ± 1.9	3.3 ± 0.0	30.7 ± 2.2	42.3 ± 0.9	47.8 ± 1.0	6.8 ± 0.2	84.7 ± 1.8	51.1 ± 0.3
16	VBG 19-016	234.0 ± 16.0	4.7 ± 0.3	30.3 ± 0.3	44.7 ± 2.0	46.0 ± 0.7	7.2 ± 0.2	89.3 ± 4.1	53.8 ± 0.5
17	VBG 19-017	187.3 ± 7.5	3.7 ± 0.2	31.7 ± 0.9	47.3 ± 3.3	47.0 ± 0.5	7.1 ± 0.2	92.0 ± 4.0	53.0 ± 1.5
18	VBG 19-018	236.7 ± 10.1	4.7 ± 0.2	31.7 ± 0.9	40.7 ± 2.9	48.8 ± 0.1	6.6 ± 0.1	81.3 ± 5.8	50.4 ± 0.5
19	VBG 19-019	163.3 ± 1.3	3.3 ± 0.0	29.3 ± 0.3	44.3 ± 2.3	48.3 ± 0.2	6.8 ± 0.1	88.7 ± 4.7	53.6 ± 0.4
20	VBG 19-020	161.7 ± 3.7	3.2 ± 0.1	31.0 ± 0.6	45.7 ± 2.7	46.4 ± 0.4	7.2 ± 0.1	90.7 ± 4.8	49.4 ± 1.5
	V. radiata (L.) Wilczek								
21	V2709-BG	162.7 ± 3.3	3.3 ± 0.1	23.3 ± 0.3	51.0 ± 1.5	33.1 ± 0.4	10.3 ± 0.1	99.3 ± 0.7	51.9 ± 1.3
22	V2802-BG	189.0 ± 5.1	3.8 ± 0.1	23.7 ± 0.3	53.0 ± 2.1	33.2 ± 0.3	10.4 ± 0.1	100.0 ± 0.0	53.4 ± 0.9
23	VGG RU 1	97.7 ± 2.0	2.0 ± 0.0	22.3 ± 0.3	51.3 ± 1.2	37.1 ± 1.8	9.3 ± 0.4	99.3 ± 0.7	48.8 ± 1.3

Table 3: Descriptive statistics of bruchid resistant traits on wild *Vigna* accessions

Traits observed	*Mean ± Standard Error*	*Min*	*Max*
Number of eggs/50 seeds (NES)	174.5 ± 9.2	59.0	270.0
Mean no. of eggs/seed (MNES)	3.5 ± 0.2	1.2	5.4
Developmental time (days) (DT)	32.7 ± 1.1	22.3	55.0
Total no. of adult emergence (AE)	34.2 ± 2.4	0.0	53.0
Mean developmental period (days) (MDP)	45.6 ± 0.7	33.1	50.0
Index of susceptibility (IS)	6.4 ± 0.3	0.0	10.4
Seed damage (%) (SD)	67.9 ± 4.7	0.0	100.0
Seed weight loss (%) (SWL)	39.1 ± 1.08	14.5	50.0

Resistance screening of *Vigna* accessions against *C. maculatus*

All the genotypes were screened under a no-choice test against pulse beetle *C. maculatus* following the assay procedure of Dongre *et al.* (1996) with some modifications as performed by Ragul *et al.* (2022). Five pairs of adults were released on 50 seeds of each genotype placed in a 15 cm diameter plastic petriplates. The insects were allowed to remain in petriplates for five days for oviposition. Adults were removed from petriplates after five days. The emerged adults were counted daily and removed from the petriplates to avoid secondary infestation. Observations were recorded as performed by Ragul *et al.* (2022) *viz*., number of eggs laid on 50 seeds (NES), ii) mean number of eggs per seed (MNES), iii) developmental time (egg + larval + pupal stages) (days) (DT), iv) total number of adult emergence (daily observation of adult emergence upto 50 Days After Infestation (DAI) (AE), v) mean developmental period (days) (MDP), vi) Howe's Index of susceptibility (IS), vii) seed damage (%) at 50 DAI (SD), viii) seed weight loss (%) at 50 DAI (SWL).

Statistical Analysis

The experiment was performed using a completely randomized design as Gomez and Gomez (1984) suggested. The descriptive statistics, including range, mean, standard error and principal component analysis (PCA) weredone by the method described by Upadhyaya *et al.* (2002). The clustering was performed using Tocher's method. The multivariate analysis was performed using software PYTHON programming language. The descriptive and association were analyzed using the statistical software TNAUSTAT statistical package (Manivannan, 2014).

Results

Characteristics of *Vigna* accessions

The qualitative and quantitative parameters of the wild *Vigna* and cultivated *Vigna* species are provided in Supplementary Table 1. The seeds of 40 *Vigna* accessions were varied concerning the seed color (black, green, brown, mottled and yellowish-green), seed shape (globose, oval and drum) and seed lustre (shiny and dull). The highest value for the hundred seed weight (7.5 g) was recorded for *V. umbellata* (IC137171-5), and *V. trilobata* recorded the lowest value (1.1 g).

Evaluation on *C. maculatus* resistance

The seeds of different *Vigna* accessions were subjected to the bioassay to assess the resistance against *C. maculatus* under a no-choice test. The resistance nature of various accessions was recorded based on the different traits and tabulated in Tables 1 and 2. All the traits were significantly different among the accessions. The oviposition on the *Vigna* accessions ranged from 59 to 270 eggs on 50 seeds. The accession *V. trilobata* (59.0 ± 1.7) recorded minimum

Table 4: Principal components of bruchid resistant traits on wild *Vigna* accessions

Traits observed	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*	*PC7*	*PC8*
Number of eggs/50 seeds (NES)	0.29	-0.48	0.42	0.05	0.02	-0.06	0.00	-0.71
Mean no. of eggs/seed (MNES)	0.29	-0.48	0.42	0.05	0.02	-0.06	0.00	0.71
Developmental time (days) (DT)	-0.37	-0.30	-0.14	0.78	-0.19	0.35	-0.02	0.00
Total no. of adult emergence (AE)	0.42	0.02	-0.32	0.18	-0.39	-0.25	0.69	0.00
Mean developmental period (days) (MDP)	-0.11	-0.61	-0.53	-0.51	-0.08	0.26	0.02	0.00
Index of susceptibility (IS)	0.40	0.26	0.17	-0.11	-0.27	0.82	0.00	0.00
Seed damage (%) (SD)	0.42	0.01	-0.33	0.15	-0.33	-0.23	-0.73	0.00
Seed weight loss (%) (SWL)	0.41	-0.02	-0.34	0.24	0.79	0.17	0.03	0.00
Eigen values	5.22	2.02	0.50	0.17	0.06	0.03	0.00	0.00
Proportion of variance	0.65	0.25	0.06	0.02	0.01	0.00	0.00	0.00
Cumulative proportion	0.65	0.91	0.97	0.99	1.00	1.00	1.00	1.00

Table 5: Clustering pattern among wild *Vigna* accessions against bruchid resistance

Cluster number	Number of genotypes	Constituent genotypes
I	9	*V. umbellate* (RED-5, RBHP-109-1, IC528870-2-36, IC528870-1-63, IC528870-6-93, IC528870-2-54, IC528870-2-78, IC528870-1-11, IC528870-4-42).
II	6	*V. glabrescens* cv. IC251372,*V. radiata*(V2709-BG, V2802-BG, VGG RU 1), *V. mungo* var. *silvestri cv.* TCR265, *V. radiata*. var. *sublobatacv.* TCR218,
III	8	*V. mungo* x *V. mungo* var. *silvestri* (VBG19-001, VBG19-002, VBG19-006, VBG19-009, VBG19-015, VBG19-017, VBG19-019, VBG19-020).
IV	12	*V. mungo* x *V. mungo* var. *silvestri* (VBG19-003, VBG19-004, VBG19-005, VBG19-007, VBG19-008, VBG19-010, VBG19-011, VBG19-012, VBG19-013, VBG19-014, VBG19-016, VBG19-018).
V	2	*V. radiata*.var. *sublobata cv.*TCR188, *V. trilobata*.
VI	3	*V. umbellata* **(**RBHP109-2, ICP1871-71, IC137171-5).

oviposition, whereas the accession VBG 19-003 (251 ± 4.6) recorded a maximum number of eggs per 50 seeds. The developmental time (*i.e.,* the first emergence of the bruchid beetles) among the accessions ranged from 22 to 55 DAI. This property of delayed emergence was observed among the accessions of *V. radiata* var. *sublobata* (TCR188) and *V. trilobata*. Complete adult emergence was observed on VBG19-005, VBN 19-009, V2709–BG, V2802-BG and VGG RU 1 before 50 DAI. In contrast, *V. radiata*. var. *sublobata* (TCR 188), *V. trilobata* and *V. umbellate* (RBHP 109-2 and IC528870-2-54) recorded less adult emergence at 50 DAI. The mean developmental period ranged from 33 to 50 days. The index of susceptibility (IS) showed that the accessions *V. radiata*. var. *sublobata* (TCR 188), *V. trilobata* and *V. umbellata* (RBHP 109-2) might be possessing resistant factors against bruchid beetles. These accessions had less seed damage and seed weight loss.

Essential components of bruchid resistance

The descriptive results of bruchid resistance traits were summarized in Table 3. The principal components were recorded and tabulated in Table 4. The number of eggs per 50 seeds ranged from 59 to 270. Mean eggs per seed ranged from 1.2 to 5.4. Developmental time among the screened genotypes ranged between 22.3 and 55.0 days. Total number of adult emergencies ranged from 0 to 53. The mean developmental period varied from 33.1 to 50.0 days after infestation. The index of susceptibility ranged from 0.0 to 10.4. Traits *viz.,* seed damage and seed weight loss ranged

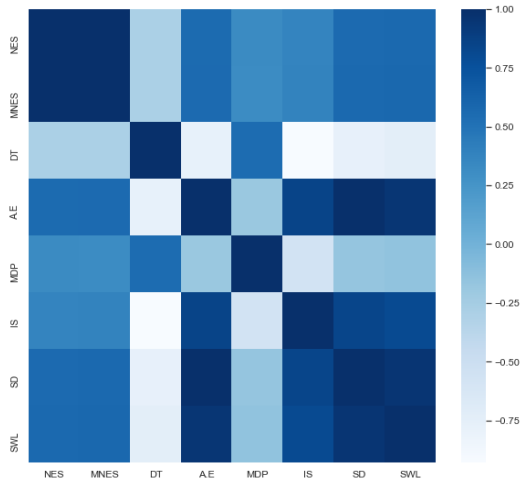


Figure 1: Correlogram heat map on various bruchid resistance traits on wild *Vigna* accessions

from 0.0 to 100.0 and 14.5 to 50.0, respectively. The principal component analysis (PCA) showed that among the eight principal components, PC1 and PC2 alone accounted for 91% of the cumulative proportion of variation (Table 4). The eigenvalues of the principal components PC1 and PC2 were more than unity (one) as per the scree plot (Supplementary Figure 1). In PC1, all the traits were positively contributed with 65% variation except traits *viz.*, development time and mean developmental period. Among them, more positive contribution was rendered by seed damage (0.42), total number of adult emergence (0.42), seed weight loss (0.41) and index of susceptibility (0.40), number of eggs per 50 seeds (0.29) and mean number of eggs per seed (0.29) (Table 4) (Supplementary Figure 2). Results of correlation coefficients for the bruchid-resistant traits are presented as Correlogram heat map in Figure 1. It indicates that trait seed weight loss had a significant positive association with seed damage (0.96), total number of adult emergence (0.95), index of susceptibility (0.81), number of eggs per 50 seeds (0.58) and mean number of eggs per seed (0.58). However, seed weight loss had recorded a significant and negative association with developmental time (-0.73) and mean developmental period (-0.14). In general, the seed weight loss, seed damage and susceptibility index had similar levels of association with other traits.

Diverse nature of *Vigna* accessions for bruchid resistance

The genetic divergence study categorized the 40 *Vigna* accessions into six clusters based on the Euclidean distance matrix under ward method (Table 5), and the dendrogram heat map is furnished in Supplementary Figure 3**.** Among the clusters, cluster IV is the major one with 12 accessions, followed by clusters I and III with nine and eight accessions, respectively. The clusters II, VI and V had 6, 3 and 2 *Vigna* accessions, respectively. Among the clusters, the accessions

in the clusters V *viz*., *V. radiata*.var. *sublobata cv.* TCR 188 and *V. trilobata* and in cluster VI *viz*., *V. umbellata cv.* RBHP 109-2, *V. umbellata cv.* ICP1871-71 and *V. umbellata cv.* IC137171-5) were recorded complete resistance to moderate resistance nature against *C. maculatus*.

Discussion

In the present investigation, 40 *Vigna* accessions comprising both wild and cultivated species were subjected to bruchid (*C. maculatus*) infestation to assess their resistance level. All the *Vigna* accessions varied both qualitatively and quantitatively based on the seed characterization. Oviposition is a critical behavior of an insect for the continuation of its race and population establishment. The seed size (in terms of 100- seed weight) and seed lusture nature does not affect the level of oviposition by the bruchid as all the seeds among the accessions were noticed for the presence of eggs on them. The bruchid resistance against the *C. maculatus* indicated that all the bruchid resistance traits significantly differed among the *Vigna* accessions. This meant that all the *Vigna* accessions differed in qualitative, quantitative and reaction towards bruchid resistance. Earlier reports indicated the influence of the qualitative and quantitative traits towards the preference of bruchid beetles through anti-xenosis properties (War *et al.,* 2017).

All the genotypes invariably showed eggs presence on every seed of the *Vigna* accessions in the study. It seems that qualitative and quantitative traits do not affect the ovipositional response level by the bruchid beetles, *i.e.,* the anti-xenosis property is not involved in this study material. Similar results were also given by Tripathi *et al.* (2015) and Ragul *et al.* (2022). Further, it is reported that the *C. maculatus* species might oviposit on any seed type that may not even be suitable for their development. For instance, the accessions *V. radiata*. var. *sublobata* **(**TCR 188), *V. trilobata* and *V. umbellata* (RBHP 109-2) were resistant against bruchid beetles as they showed less index of susceptibility, less adult emergence, prolonged mean developmental period, less seed damage and seed weight loss, yet they were found with eggs. The resistant nature among the accessions is due to the property delayed emergence of adults. Somta *et al.* (2008) and Ragul *et al.* (2022) also reported the property of delayed emergence property. Complete adult emergence was observed on some of the accessions *viz.,* VBG19-005, VBN 19-009, V2709–BG, V2802-BG and VGG RU 1 before 50DAI. V2709–BG and V2802-BG were previously identified as resistant sources (Talekar and Lin, 1992; Somta *et al*., 2007). However, these accessions were recorded as susceptible in this study. Among wild *Vigna* species, *V. radiata*.var. *sublobata, V. umbellata and V. trilobata* accessions were reported earlier for their moderate to complete resistance to bruchid beetles (Chen *et al.,* 2013; Seram *et al.,* 2016, Aidbhavi *et al.,* 2021).

Principal component studies and correlation analysis indicated that the traits *viz.,* seed damage, total number of adult emergence, seed weight loss and index of susceptibility recorded more positive contributions towards the variation and are more correlated among them. Hence, those traits with more contribution towards variation are most important when selecting parental lines for bruchid resistance. Therefore, these traits can be directly involved in a genetic improvement programme. Further, the resistant accessions were grouped under clusters V and VI based on the divergence study may be utilized in the future crop improvement programme to impart resistance to *C. maculatus.*

Conclusion

The present experimental results provided the resistance level among the wild *Vigna* accessions and cultivated *Vigna* species against pulse beetle *i.e., C. maculatus.* Based on the experiment, the accessions *viz., V. radiata*. var. *sublobata* **(**TCR 188), *V. trilobata* and *V. umbellata* (RBHP 109-2, ICP1871-71, IC137171-5) were found as resistant towards *C. maculatus*. They were also confirmed resistance based on the critical component traits. Hence these accessions would serve as an excellent resistance source in framing resistance breeding programme against *C. maculatus*.

References

Anonymous (2019) Project Coordinator Report- (2018-19) All India Coordinated Research Project on MULLaRP, ICAR- Indian Institute of Pulses Research, Kanpur-208204, Uttar Pradesh, India, Pp-46.

Aidbhavi R, A Pratap, P Verma, A Lamichaney, SM Bandi, SD Nitesh,M Akram, MRathore, B Singh and NP Singh (2021) Screening of endemic wild Vigna accessions for resistance to three bruchid species. *J. Stored Prod. Res.* **93**: p.101864.

Asif M, LW Rooney, R Aliand MNRiaz (2013) Application and opportunities of pulses in food system – a review. *Critical Rev. Food Sci. Nutrition* **53**: 1168–1179.

Began M, JL Harper and CR Townsend (1990) Ecology, second ed. Blackwell, Oxford, 0632013370. 945pp

Chen HM, HM Ku, R Schafleitner, TSBains, CG Kuo and CA Liu (2013) The major quantitative trait locus for mungbean yellow mosaic Indian virus resistance is tightly linked in repulsion phase to the major bruchid resistance locus in a cross between mungbean [*Vigna radiata* (L.) Wilczek] and its wild relative *Vigna radiata* ssp. *sublobata*. *Euphytica.* **192**: 205–216. *https://doi.org/10.1007/s10681-012-0831- 9.*

Credland PF (1994) Bioassays with bruchid beetles: problems and (some) solutions. Proceedings of the 6th International Working Conference on Stored-Product Protection. CAB International, Canberra, Australia, ISBN 0851989322, pp. 17–23. Wallingford, United Kingdom

Dongre T, S Pawar, R Thakare and M Harwalkar (1996) Identification of resistant sources to cowpea weevil (*Callosobruchus maculatus* (F)) in *Vigna sp.* and inheritance of their resistance in black gram (*Vigna mungo* var. *mungo*). *J. Stored Prod. Res.* **32(3)**: 201-204

Duraimurugan P, K Raja andA Regupathy (2011) An eco-friendly approach for management of pulse beetle, *Callosobruchus maculatus* through neem formulations assisted with pitfall trap. *J. Food Leg.* **24(1)**: 23-27

Gomez KA and AA Gomez(1984) Statistical procedures for agricultural research: John Wiley & Sons

Harlan JR(1984) Evaluation of wild relatives of crop plants In: J.H.W. Holden & J.T. Williams (Eds.), *Crop Genetic Resources Conservation and Evaluation*, pp 212–222. George Allen & Unwin, London, U.K.

Kashiwaba K, N Tomooka, A Kaga, OK Han and DA Vaughan (2003) Characterization of resistance to three bruchid species (*Callosobruchus* spp. Coleoptera: bruchidae) in cultivated rice bean (*Vigna umbellata*). *J. Econ.Ento.* **96**: 207–213. *https://doi.org/10.1093/jee/96.1.207*.

Lawn RJ (1995) The Asiatic *Vigna* species. In: J. Smartt& N.W. Simmonds (Eds.), *The* Evolution of Crop Plants, pp. 321–326. 2nd edition. Longman, Harlow, U.K

Manivannan N (2014) TNAUSTAT- Statistical package. Retrieved from *https://sites.google.com/site/tnaustat*

Mishra SK, MLR Macedo, SK Panda and J Panigarhi (2017) Bruchid pest management in pulses: past practices, present status and use of modern breeding tools for development of resistant varieties. *Annals. Appl. Bio.* **172(1)**: 4–19. *https://doi.org/ 10.1111/aab.12401*

Nair RM, AK Pandey, AR War, H Bindumadhava, T Shwe, AKMM Alam, A Pratap, SR Malik, R Karimi, EK Mbeyagala, CA Douglas, J Rane and R Schafleitener(2019) Biotic and abiotic constraints in mungbean production - progress in genetic improvement. *Frontiers. Pl. Sci.* **10**: 1340. *https://doi.org/10.3389/fpls.2019.01340*.

Pratap A, C Douglas,U Prajapati, G Kumari, AR Was, R Tomar, AK Pandey and S Dubey(2020) Breeding progress and future challenges: biotic stresses. In: Nair, R., Schafleitner, R., Lee, S.H. (Eds.), The Mungbean Genome. Compendium of Plant Genomes. Springer, Cham. *https://doi.org/10.1007/978-3-030-20008-4_5*.

Pratap A and SK Gupta (2009) Biotechnological interventions in host plant resistance In: Peshin, Rajinder, Dhawan, Ashok K. (Eds.), Integrated Pest Management: Innovation-Development Process. Springer Publishers, Dordrecht, UK. *https://doi. org/10.1007/978-1-4020-8992-3, 183–207pp*.

Ragul S, N Manivannan, K Iyanar, N Ganapathy and GKarthikeyan (2022) Screening and biochemical analysis on blackgram genotypes for resistance against storage pest bruchid (*Callosobruchus maculatus* (F.)). *Legume res.* **45(3)**: 371 – 378. *doi: 10.18805/LR-4528*.

Sarkar S, S Ghosh, M Chatterjee, P Das, T Lahari, A Maji, N Mondal, KK Pradhan and S Bhattacharyya (2011) Molecular markers linked with bruchid resistance in *Vigna radiata* var. *sublobata* and their validation. *J. Pl.Biochem.Biotech.* **20**: 155–160. *https://doi.org/10.1007/s13562-011-0039-4*.

Seram D, N Senthil, M Pandiyan and J Kennedy (2016) Resistance determination of a South Indian bruchid strain against rice bean landraces of Manipur (India). *J. Stored Prod. Res.* **69**: 199–206. *https://doi.org/10.1016/j.jspr.2016.08.008*.

Somta P, C Ammaranan, PACOoi and PSrinives (2007) Inheritance of seed resistance to bruchids in cultivated mungbean (*Vigna radiata* L. Wilczek). *Euphytica* **155**: 47–55.

Somta P, W Musch, B Kongsamai, S Chanprame, S Nakasathien, T Toojinda, W Sorajjapinun, WSeehalak, S Tragoonrung and P Srinives (2008) New microsatellite markers isolated from mungbean (*Vigna radiata* (L.) Wilczek). *Mol. eco.Resour.* ***8*(5)**: 1155-1157.

Souframanien J, SK Gupta and Y Gopalakrishna (2010) Identification of quantitative trait loci for bruchid (*Callosobruchus maculatus*) resistance in black gram [*Vigna mungo* (L.) hepper]. *Euphytica* **176**: 349–356. *https://doi.org/10.1007/s10681-010- 0210-3*.

Subramaniyan R, M Narayana, I Krishnamoorthy, G Natarajan and K Gandhi (2021) Mapping and mining of major genomic regions conferring resistance to Bruchid (*Callosobruchus maculatus*) in blackgram (*Vigna mungo*). *Pl. Breed.* **140**(**5**): 896-906.

Sun L, XZ Chen, SH Wang, LX Wang, CY Liu, L Mei and XU Ning (2008) Heredity analysis and gene mapping of bruchid resistance of a mungbean cultivar V2709. *Agricultural Sciences in China* **7**: 672–677. *https://doi.org/10.1016/S1671-2927(08)60101-7*.

Talekar NS and CP Lin (1992) Characterization of *Callosobruchus chinensis* (Coleoptera: Bruchidae) resistance in mungbean. *J. Eco.Ento.* **85**: 1150–1153

Tateishi Y (1985) A revision of the Azuki bean group, the subgenus *Ceratotropis*of the genus *Vigna* (Leguminosae). Ph. D Dissertation, Tohoku University, Japan. Pp: 292

Tomooka N, K Kashiwaba, DAVaugham, M Ishimotoand Y Egawa (2000) The effectiveness of evaluating wild species: searching for sources of resistance to bruchid beetles in the genus *Vigna* subgenus *ceratotropis*. *Euphytica* **115**: 27–41. *https://doi.org/10.1023/A:1003906715119*.

Tripathi K, SK Chauhan, PG Gore, T Prasad, K Srinivasanand S Bhalla (2015) Screening of cowpea [*Vigna unguiculata* (L.) Walp.] accessions against pulse-beetle, *Callosobruchus chinensis* (L.). *Legume Res.*- ***38*(5)**: 675-680.

Upadhyaya HD, R Ortiz,PJ Bramel andS Singh(2002) Phenotypic diversity for morphological and agronomic characteristics in chickpea core collection. *Euphytica* **123**: 333-342.

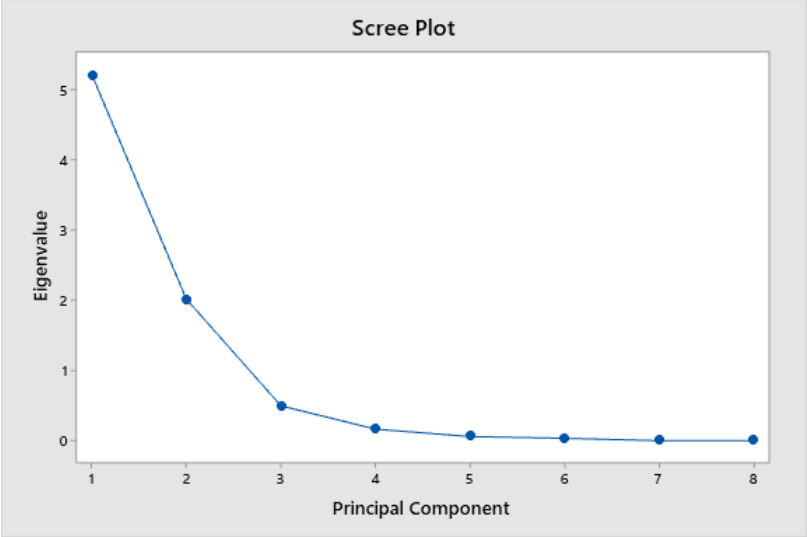
War AR, S Murugesan, VN Boddepalli, R Srinivasan and RM Nair (2017) Mechanism of resistance in mungbean [*Vigna radiata* (L.) R. Wilczek var. *radiata*] to bruchids, *Callosobruchus* spp. (Coleoptera: bruchidae). *Frontiers. Pl. Sci.* **8**: 1031. *https://doi.org/10.3389/fpls.2017.01031*.

Xu RQ, N Tomooka, DA Vaughanand K Doi (2000) The *Vigna angularis* complex: genetic variation and relationships revealedby RAPD analysis, and their implications for in-situ conservationand domestication. *Genet. Resour. Crop Evol.* **47**: 123-134

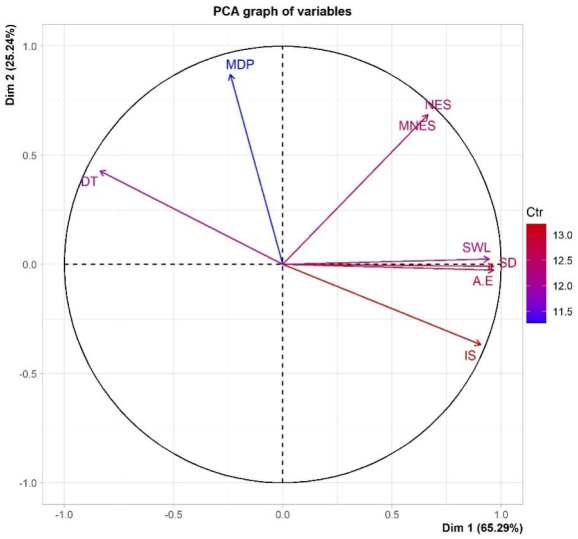
Supplementary Table 1: Description of 40 wild and cultivated *Vigna* accessions involved in the study

S. No	*Vigna species*	*Accession ID*	*Source*	*Seed colour*	*Seed shape*	*Seed lustre*	*100- Seed weight (g) ± SE*
1	*V. glabrescens* (Maréchal *et al.*)	IC251372	NPRC, TAMILNADU	Black	Oval	Shiny	3.3 ± 0.2
2	*V. mungo* var. *silvestris*	TCR265	NPRC, TAMILNADU	Green	Oval	Shiny	1.5 ± 0.0
3	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-001	NPRC, TAMILNADU	Brown	Oval	Shiny	4.7 ± 0.1
4	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-002	NPRC, TAMILNADU	Black	Globose	Dull	4.1 ± 0.1
5	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-003	NPRC, TAMILNADU	Black	Globose	Dull	3.8 ± 0.0
6	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-004	NPRC, TAMILNADU	Brown	Globose	Dull	4.6 ± 0.1
7	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-005	NPRC, TAMILNADU	Brown	Globose	Dull	4.6 ± 0.1
8	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-006	NPRC, TAMILNADU	Brown	Globose	Dull	3.9 ± 0.0
9	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-007	NPRC, TAMILNADU	Brown	Globose	Dull	4.6 ± 0.1
10	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-008	NPRC, TAMILNADU	Black	Globose	Dull	4.6 ± 0.2
11	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-009	NPRC, TAMILNADU	Brown	Globose	Dull	4.3 ± 0.1
12	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-010	NPRC, TAMILNADU	Black	Oval	Dull	3.7 ± 0.1
13	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-011	NPRC, TAMILNADU	Brown	Globose	Dull	4.4 ± 0.1
14	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-012	NPRC, TAMILNADU	Brown	Globose	Dull	4.6 ± 0.1
15	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-013	NPRC, TAMILNADU	Black	Globose	Dull	4.5 ± 0.1
16	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-014	NPRC, TAMILNADU	Brown	Oval	Dull	4.8 ± 0.3
17	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-015	NPRC, TAMILNADU	Brown	Globose	Dull	4.4 ± 0.1
18	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-016	NPRC, TAMILNADU	Black	Oval	Dull	4.4 ± 0.2
19	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-017	NPRC, TAMILNADU	Black	Globose	Dull	4.0 ± 0.0
20	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-018	NPRC, TAMILNADU	Black	Oval	Dull	4.8 ± 0.2
21	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-019	NPRC, TAMILNADU	Mottled	Globose	Dull	4.3 ± 0.0
22	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-020	NPRC, TAMILNADU	Mottled	Globose	Dull	4.8 ± 0.3
23	*V. radiata* (L.) Wilczek	V2709-BG	AVRDC, TAIWAN	Green	Globose	Shiny	3.5 ± 0.1
24	*V. radiata* (L.) Wilczek	V2802-BG	AVRDC, TAIWAN	Green	Globose	Dull	3.7 ± 0.0
25	*V. radiata* (L.) Wilczek	VGG RU 1	NPRC, TAMILNADU	Green	Globose	Shiny	3.6 ± 0.0
26	*V. radiata*.var. *sublobata* (Roxb.) Verdcourt	TCR218	NPRC, TAMILNADU	Mottled	Oval	Dull	1.4 ± 0.0
27	*V. radiata*.var. *sublobata* (Roxb.) Verdcourt	TCR188	NPRC, TAMILNADU	Green	Oval	Shiny	1.8 ± 0.0
28	V. trilobata	Local accession	NPRC, TAMILNADU	Black	Oval	Dull	1.1 ± 0.0
29	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	IC528870-2-36	NPRC, TAMILNADU	Brown	Drum	Dull	4.2 ± 0.1
30	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	IC528870-1-63	NPRC, TAMILNADU	Green	Drum	Dull	3.9 ± 0.0
31	*V. umbellata* (Thunb.) Ohwi&H. Ohashi	IC528870-6-93	NPRC, TAMILNADU	Green	Drum	Shiny	3.9 ± 0.1
32	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	IC528870-2-54	NPRC, TAMILNADU	Brown	Drum	Shiny	3.8 ± 0.0
33	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	IC528870-2-78	NPRC, TAMILNADU	Brown	Drum	Shiny	3.3 ± 0.2
34	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	ICP1871-71	NPRC, TAMILNADU	Mottled	Drum	Dull	5.5 ± 0.1

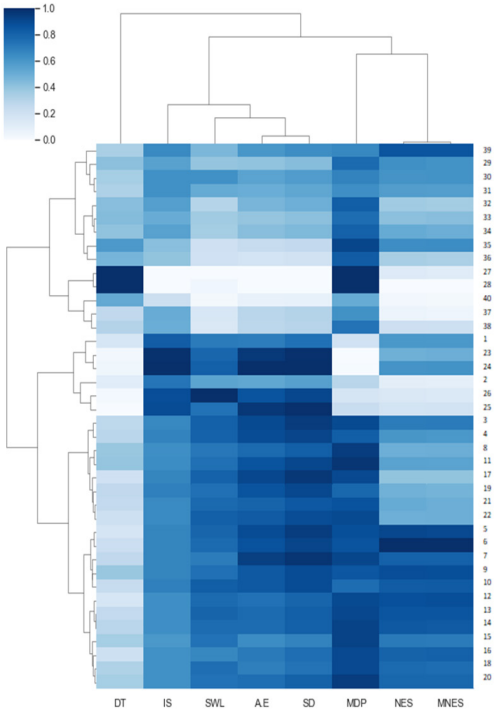
35	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	IC137171-5	NPRC, TAMILNADU	Yellowish green	Drum	Shiny	7.5	±	0.0
36	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	IC528870-1-11	NPRC, TAMILNADU	Brown	Drum	Shiny	5.9	±	0.0
37	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	IC528870-4-42	NPRC, TAMILNADU	Yellowish green	Drum	Dull	3.6	±	0.1
38	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	RBHP-109-1	NPRC, TAMILNADU	Brown	Drum	Dull	5.9	±	0.1
39	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	RED-5	NPRC, TAMILNADU	Dark brown	Drum	Shiny	4.9	±	0.1
40	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	RBHP 109-2	NPRC, TAMILNADU	Mottled	Drum	Shiny	5.3	±	0.0



Supplementary Figure 1: Scree plot for the eigenvalues of the principal components on bruchid infestation



Supplementary Figure 2: Biplot on various bruchid resistance trait on wild and cultivated *Vigna* accessions



Supplementary Figure 3: Dendrogram heat map of various bruchid resistance traits on wild and cultivated *Vigna* accession

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.06

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Biophysical and Morphological Basis of Resistance against Linseed (*Linum usitatissimum* L.) Bud fly (*Dasyneura lini* Barnes)

Vinay Kumar[1], Gautham S[2], Kavita Gupta[3*], V.K. Biradar[4], Vikender Kaur[3], Beena Nair[4] and Ajay Kumar[1]

Abstract

A field experiment was conducted at the research cum instructional farm, College of Agriculture, Nagpur, Dr. PDKV, Maharashtra during *rabi* 2021-22 with the objective to find the biophysical and morphological basis of resistance against linseed bud fly (*Dasyneura lini* Barnes). A set of 36 germplasm was sown in RBD in paired rows of 2 m and a spacing of 45 cm x 10 cm. The results indicated that resistant germplasm against bud fly IC498428 and Jawahar-17 had relatively higher sepal thickness (21.18 and 19.64 µ) as well as lower average infestation recorded (8.19 and 9.75%) while susceptible germplasm IC413173 (49.12%), IC498783 (42.70%), IC498924 (42.48%) and EC011748 (45.00%) exhibited minimum sepal thickness (6.90, 9.28, 9.32 and 8.33 µ, respectively). The character association studies showed a highly significant negative correlation (r = -0.852**) between the thickness of sepals and the percentage of bud fly infestation. The correlation between plant height and bud infestation was negative (-0.042) and non-significant.

Keywords: Bud fly, Linseed, Sepal thickness, Susceptible, Resistance.

[1]Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India.

[2]Division of Genetics, ICAR- Indian Agriculture Research Institute, New Delhi, India.

[3]ICAR- National Bureau of Plant Genetic Resources, New Delhi, India.

[4]PDKV- College of Agriculture, Nagpur, Maharashtra, India.

***Author for correspondence:**

kavita.gupta@icar.gov.in

Received: 28/12/2022 **Revised:** 01/03/2023

Accepted: 20/04/2023

How to cite this article: Kumar, V., Gautham, S., Gupta, K., Biradar, V.K., Kaur, V., Nair, B., Kumar, A. (2023). Biophysical and Morphological Basis of Resistance against Linseed (*Linum usitatissimum* L.) Bud fly (*Dasyneura lini* Barnes). Indian J. Plant Genetic Resources. 36(3), 382-386. **DOI:** 10.61949/0976-1926.2023.v36i03.06

Introduction

Linseed (*Linum usitatissimum L.*) belongs to family Linaceae, which is grown worldwide for its multifunctional properties like food, fiber, and fuel as a *rabi* season crop. Due to its high adaptability, linseed is India's fourth major oil seed crop and third major fiber crop. It plays a vital role in industries as its linen, which is costlier than cotton fiber, is used as a raw material for making clothes, paper cigarette filter, packaging material and other eco-friendly products. Linseed fibre, obtained from the stem of plant, is more tensile than cotton.

Due to its strength, non-elasticity, repeatability and recyclable nature, it is very attractive to be used as rope and thread, which is the major interest for its cultivation (Jhala and Hall 2010). After oil extraction, the cake is used for cattle as a protein stock and fine manure (Sankari, 2000; Kurt and Bozkurt, 2006). Different names know it at different locations like Arasi, Alsi, Jawas, Tisi and Chana. Linseed oil makes several daily use products, such as paint, varnishes, water-resistant fabric and linoleum (Dash *et al*., 2017). Linseed is the best herbal source of PUFA- Poly unsaturated fatty acids such as linoleic and linolenic (ω-3) acid, which are essential for humans since they can't be synthesized in the body. Besides the highly desirable ω-6/ ω-3 fatty acid ratio of 0.3:1, linseed is also a rich source of secoisolariciresinol diglucoside (SDG) and a major bioactive lignan, which provides protection against certain types of cancers and hormonal disorders.

Linseed contains 33 to 42% seed oil and 18 to 23% protein. India contributes to 4.41 and 3.66% of area and production, respectively (Statistics of Directorate of Oilseed Development, Ministry of Agriculture and Farmers Welfare, Govt. of India, 2020). According to Srivastava (2009), linseed is grown mostly under rainfed (63%), *utera* (25%) and irrigated (17%) and input-starved conditions in major linseed-producing states, such as Madhya Pradesh, Chhattisgarh, Maharashtra, Jharkhand, Uttar Pradesh, and Odisha. In India, the net sown area of linseed crop is 1.83 lakh ha. production is 1.11 lakh tonnes during 2021-22 (FAOSTAT, 2022) with a 2.5% growth rate and an average 604 kg/ha yield. in India. Linseed is still considered an oilseed crop with unexplored potential for commercial production concerning industrial, nutritional and nutraceutical utility.

Various biotic and abiotic factors are responsible for adverse effects of crop yield and bud fly is a major destructive pest of linseed, which has been reported to cause economic loss ranging from 37 to 80% (Malik, 2006). Among the factors that influence changes in population densities of bud fly are seasonal effects of weather, ongoing changes in climatic conditions and mechanisms through which they act upon its population density (Karuppaiah and Sujayanad, 2012). Bud fly (*Dasyneura lini* Barnes) is a dipteran fly belonging to the Cecidomyidae family and is a slow-flying insect. Therefore, to stabilize the yield and reduce the bud fly infestation on the basis of host plant resistant potentiality of genotypes, this research work evaluating for resistant genotypes of linseed under field conditions was taken up to identify the probable resistance host plant to bud fly, which could possibly help in improving yield and eventually the farmers' income.

Material and Methods

A field experiment was conducted at a research farm, College of Agriculture, Nagpur (Dr. PDKV, Akola). A set of 36 germplasm (Including two checks) were sown in randomized block design in paired row of 2 m row length and spacing was maintained at 45 x 10 cm in last week of November 2021 during *rabi* season for screening against linseed bud fly. Agronomic practices were maintained as per crop recommendation (Braidek, 1975) and no plant protection measures were given. After every 10 entries, Neelum (Susceptible check) and Neela (Resistant check) were sown. Bud fly incidence was recorded at the dough stage of crop.

Table 1: Classification of bud fly infestation range

Sl. No.	*Bud infestation (%)*	*Category*
1.	0–10	Highly resistant
2.	10.1–25	Moderate resistant
3.	25.1–40	Moderate susceptible
4.	40.1 60	Susceptible
5.	>60	Highly susceptible

Three plants were selected per entry for observation, followed by counting their total number of flower buds and infested buds. Bud fly Infestation Index (BII) was calculated as the percentage of infected buds among total number of buds and statistical methods such as, descriptive statistics, Pearson's Correlation coefficient, phenotypic path analysis etc. were used to establish relationships among characters. The germplasm was taken from the National Gene Bank of ICAR- National Bureau of Plant Genetic Resources, New Delhi

Table 2: Influence of sepal thickness on bud fly infestation

Sl. No.	*Accession number*	*Sepal thickness (µ m)*			*Mean (µ m)*	*Average bud fly infestation (%)*
		R1	*R2*	*R3*		
1	IC598324	18.61	15.88	19.12	17.87	15.69
2	EC115148	16.56	14.24	16.14	15.65	18.37
3	IC564606	17.49	17.39	18.6	17.83	18.01
4	IC525982	15.88	20.89	20.1	18.96	17.00
5	IC597268	10.18	9.62	12.55	10.78	29.15
6	IC498428	22.29	19.39	21.86	21.18	8.19
7	IC585268	13.07	16.11	18.6	15.93	19.39
8	IC629184	14.09	13.59	20.12	15.93	17.23
9	EC041535	9.21	10.88	10.98	10.36	30.40
10	IC096652	8.08	7.68	7.11	7.62	38.98
11	IC526030	15.9	21.75	18.93	18.86	25.75
12	IC525936	10.44	8.57	10.82	9.94	28.99
13	IC585341	10.26	9.6	10.09	9.98	29.57
14	IC096500	10.39	11.69	9.97	10.68	28.16
15	IC499135	21.64	21.04	22.26	21.65	17.76
16	IC525950	19.06	19.14	16.18	18.13	25.54
17	IC498924	8.15	9.13	10.67	9.32	42.48
18	EC541203	15.42	13.75	14.66	14.61	25.12
19	EC022872	10.1	8.98	9.31	9.46	30.70
20	IC621685	18.44	16.4	23.69	19.51	25.64
21	IC499139	17.38	12.7	20.25	16.78	25.00
22	IC526153	16.49	22.51	15.46	18.15	19.53
23	IC498738	14.29	15.21	16.43	15.31	26.32
24	IC498783	9.22	8.78	9.83	9.28	42.70
25	IC096558	11.18	10.21	9.59	10.33	20.77
26	IC498596	15.57	15.87	16.56	16.00	22.12
27	IC413173	6.58	5.38	8.75	6.90	49.12
28	EC541211	10.62	9.53	10.44	10.20	30.58
29	IC510949	21.81	16.43	15.38	17.87	16.44
30	Jawahar-17	22.12	19.23	17.58	19.64	9.75
31	IC498975	13.41	13.67	13.56	13.55	20.93
32	IC498991	12.24	12.39	11.93	12.19	19.91
33	EC011748	7.42	8.44	9.14	8.33	45.00
34	IC526028	12.75	12.66	15.81	13.74	22.64
35	Neelum	9.46	11.3	10.54	10.43	36.75
36	Neela	25.15	22.62	17.51	21.76	5.61

Table 3: Categorization of 36 germplasm based on pest infestation

Sl. No.	*Infestation range (%)*	*Category*	*No. of accessions*	*Germplasm*
1	0–10	Highly resistant	3	Jawahar-17, IC498428 and Neela (Check)
2	10.1–25	Moderate resistant	14	IC598324, EC115148, IC564606, IC525982, IC585268, IC629184, IC499135, IC526153, IC096558, IC498596, IC510949, IC498975, IC498991, IC526028.
3	25.1–40	Moderate susceptible	15	IC597268, EC041535, IC096652, IC526030, IC525936, IC585341, IC096500, IC525950, EC541203, EC022872, IC621685, IC499139, IC498738, EC541211 and Neelum (Check).
4	40.1–60	Susceptible	4	IC498924, IC498783, IC413173 and EC011748.
5	>60	Highly susceptible	0	Nil

for the experimental trial. The germplasm was categorized into five groups (Malik, 2005) as depicted in Table 1.

Biophysical and Morphological Basis of Resistance

The response shown by plants against insect pests can be attributed to morphological or biophysical traits. Selected entries of linseed were investigated on the basis of biophysical and morphological parameters like plant height and sepal thickness under field conditions. During the maturity stage, plant height was measured in centimeters, from the soil surface (base of the plant) to apical portion of the plant using a standard scale to determine if height plays a role in resistance.

Sepal thickness were studied under laboratory conditions and correlated with bud infestation. Thirty-six germplasm lines were selected, including Neelum susceptible and Neela resistant checks. Three pre-mature buds per line were taken from three different plants for the purpose of measuring sepal thickness. Selected buds were peeled out and cut the cross sections of sepals with the help of sharp knife were measured using a trinocular microscope in micrometers (µm) given in Table 2.

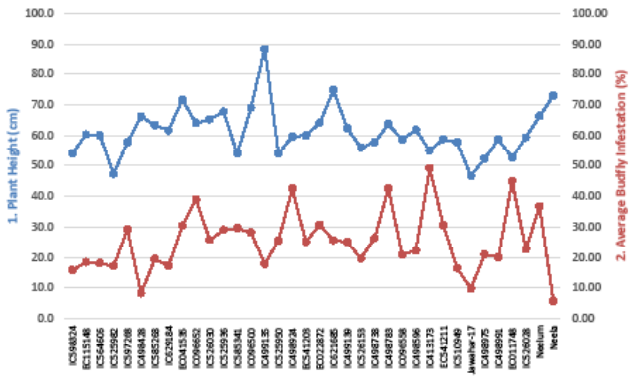


Figure 1: Plant height against average bud fly infestation of 36 germplasms

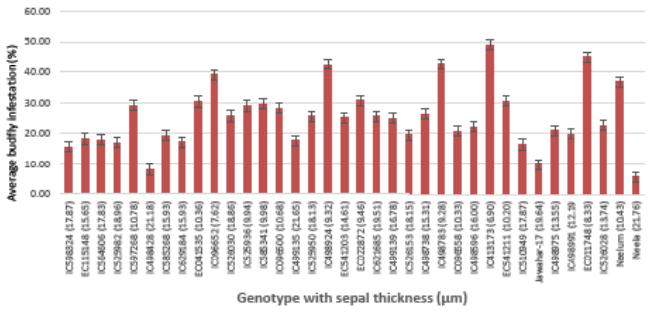


Figure 2: Average bud fly infestation on the basis of sepal thickness

Result and Discussions

The bud fly gradually increased in population from the bud initiation stage to dough stage of crop, but maximum bud fly population was seen from second fortnight of February to first week of March. Thirty-six linseed germplasm were screened against bud fly infestation under natural conditions. The bud fly infestation ranged between 8.19% (IC498428) and 49.12% (IC413173). The germplasm had different degrees of susceptibility to bud fly attack and was classified accordingly (Malik, 2005) Table 1. Out of 36 germplasms, only two germplasm were recorded for minimum average infestation of bud fly that came under resistant category (0.00–10.00%), while 14 germplasm came under moderately resistant category (10.01–25%) against bud fly infestation. Fifteen germplasm were grouped under the moderately susceptible category, having 25.01 to 40% infestation. Four linseed germplasm lines had 40.01 to 60.00% bud fly infestation and were categorized under the susceptible category. There was no germplasm found within the highly susceptible category (>60% infestation) during the investigation period depicted in Table 3.

In the experiment, recorded plant height ranged between 47.27 cm in IC525982 and 88.33 cm in IC499135 and the mean of plant height was recorded at 61.22 cm. The correlation between plant height and bud fly infestation was negative (-0.042) and non-significant (Figure 1). The result was contradictory to studies by Ekka (2017) and Gupta (2011), where the plant height showed a positive non-significant correlation with bud fly infestation (0.233 and 0.028, respectively).

Sepal thickness is a very important factor associated with histological resistance in linseed against linseed bud fly. The present investigation showed the bud fly intensity with

the flower bud sepal thickness relationship. On the basis of experiments, germplasm with maximum sepal thickness was IC498428 and Jawahar-17 (21.18 and 19.64 µ, respectively), which showed strong resistance against bud fly infestation (8.19 and 9.755, respectively). Among 36 germplasms, IC413173 had minimum sepal thickness (6.90 µ), and the highest bud fly infestation (49.12%). The thickness of sepal in another germplasm ranged between 6.90 and 23.43 µ. The relationship of the sepal thickness with bud infestation indicates that resistant germplasm, Neela, IC498428 and Jawahar-17 had maximum sepal thickness 23.43, 21.18 and 19.64 µ, respectively and minimum bud infestation 5.61, 8.19 and 9.75%, respectively. Among 36 linseed germplasm only four germplasm IC413173, EC011748, IC498783 and IC498924 suffered maximum infestation i.e., 49.12, 45.00, 42.70, and 42.48%, respectively, which had sepal thickness 6.90, 8.33, 9.28 and 9.32 µ (Figure 2). Statistical correlation between sepal thickness and bud fly was found to be negatively correlated (-0.852**) phenotypic path coefficient of -0.3898 and r value of -0.797**. From the negatively significant value of correlation coefficient between sepal thickness and bud fly infestation, it is clear that when sepal thickness increases, the infestation of bud fly decreases. Similar findings were reported by Ekka (2017) and Gupta (2015). Pal and Malik (2020), who studied the impact of sepal thickness on bud fly infestation, reported negative non-significant correlation (r= -0.7224) between the characters. Our findings reflect significantly negatively correlation (-0.852**) between sepal thickness and bud fly infestation. Among the biophysical parameters, sepal thickness played an important role in bud fly infestation. Genotypes with less thickness of sepal are favorable for bud fly infestation and vice versa.

Conclusion

Among the 36 germplasm IC498428 (8.19% infestation) and Jawahar-17 (9.75% infestation) were found resistant to bud fly. The correlation studies showed that there was negative significant correlation between sepal thickness and bud fly infestation (-0.852**). Hence, genotypes with thick sepals exhibit more resistance against bud fly infestation. Furthermore, tall genotypes showed higher resistance as plant height was negatively correlated with bud fly infestation (-0.042).

Acknowledgment

This work was conducted as a part of post-graduate thesis programme for the course M.Sc. (Ag) in Agriculture Entomology under the guidance of the corresponding author. The authors would like to acknowledge the ICAR-National Bureau of Plant Genetic Resources, New Delhi and College of Agriculture, Nagpur for technical assistance for the smooth conduction and completion of this research work.

References

Biradar SA, K Ajith Kumar, B Rajanna, AS Savitha, GV Shubha, I Shankergoud, BM Chittapur, and PK Singh (2016) Prospects and challenges in linseed (*Linum usitatissimum* L.) production. A review. *J. Oilseeds Res*. **33(1)**: 1-13.

Biradar VK, AY Thakare and PP Deshpande (2017) Determination of economic threshold level of linseed bud fly (*Dasyneura lini* Barnes). *J Ecol Environ*. **35(2)**: 774-777.

Braidek, JG (1975) Agronomic considerations in flax production. In: Harapiak, J.T. (Ed.). Oil Seed and Pulse Crops in Western Canada: A Symposium. Western Co-operative Fertilizers, Calgary, Alberta, pp. 279–285.

Dash1 J, BS Naik and UB Mohapatra (2017) Research article; Linseed: a valuable crop plant. *Int. J. Adv. Res.* (IJAR). **5(3)**: 1428-1442.

Deole S, AK Gupta and D Dash (2015) Neem-based formulations used against bud fly, *Dasyneura lini* Barnes in Linseed (*Linum usitatissimum*) crop. *Int. J. Sci. Res. Publ.* **5(2)**: 1-4.

Ekka RK, S Mandal, RS Meena and S Padamshali (2018) Some physical basis of host plant resistance in linseed (*Linum usitatissimum* L.), against bud fly, *Dasyneura lini* (Barnes). *J. Pharmacogn. Phytochem*. pp: 2010-2015.

Ekka RK (2017) Studies on seasonal incidence and screening of various germplasm of linseed against linseed bud fly Dasyneura lini Branes. M.Sc. (Ag.) thesis, Banaras Hindu University, Varanasi, 43p.

FAO. 2021. World Food and Agriculture - Statistical Yearbook 2021. Rome.https://doi.org/10.4060/cb4477en.

Farrukh A, AB Lanjar, BH Chang, FH Magsi, K Asghar, A Rajput, AD Keerio and LB Qureshi (2017) Insect biodiversity in different varieties of linseed. *Eur. J. Acad. Res*. **4**: 8306-8335.

Gupta Avinash Kumar (2011) Basis of Resistance in Linseed (Linum usitatissimum Linn) Against Linseed Bud Fly (Dasyneura lini Barnes). Thesis submitted to Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India. pp 69-89.

Gupta MP (2004) Field screening of linseed (*Linum usitatissimum*) Linn. Genotype of bud fly (*Dasyneura lini* Barnes). *J. Oilseeds Res*. **21(1)**: 108-111.

Jhala AJ and MH Linda (2010) Flax (*Linum usitatissimum* L.): Current uses and future applications. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, T6G 2P5, AB, Canada. *Aust. J. Basic Appl. Sci.* **4(9)**: 4304-4312.

Kaur V, S Kumar, R Yadav, DP Wankhede, J Arvind, J Radhamani, JC Rana and A Kumar (2018) Analysis of genetic diversity in Indian and exotic linseed germplasm and identification of trait specific superior accessions. *J. Environ. Biol.* **39**: 702-709.

Kaur V, SK Yadav, Sheela, D Singh, V Kumar, J Arvind and A Kumar (2022) Screening of linseed germplasm collection conserved in Indian National Gene Bank for drought tolerance and identification of trait specific promising accessions. *Int. Conf. on AAFS Aug. 22 - 24th, 2022*. pp: 146.

Kaur V, R Yadav and DP Wankhede (2017) Linseed genetic resources for climate change intervention and future breeding. *J. Appl. Nat. Sci*. **9**: 1112-1118.

Karuppaiah, V and GK Sujayanad (2012) Impact of climate change on population dynamics of insect pests. World. *J. Agric. Sci.* **8(3)**: 240-246.

Kumar P, P Kumar and HB Sharma (2021) Management of linseed bud fly (*Dasineura lini* Barnes) effect and proper use of insecticides. *Int. J. Res. Eng. Appl. Manag.* (IJREAM). **7(4)**:

2454-9150.

Kumar V, D Singh, B Jat and V Kaur (2022) Linseed: Essential Field Practices and Cultivation Techniques. *Agricolation Article* ID: AG25. **2(4)**: 1-5.

Kunal G, TN Anil, RB Goswami, P Nirala and SS Acharya (2019) Insect herbivores and their natural enemies in linseed and their population dynamics. *Indian J. Entomol.* **81(3)**: 458-462.

Malik YP (2005) Estimation of economic threshold level for bud fly (*Dasyneura lini* Barnes) in linseed (*Linum usitatissimum* L.). *J. Oilseeds Res*. **22(1)**: 100-102.

Minz PK, R Prasad and D Prasad (2009) Varietal screening of linseed against bud fly (*Dasyneura lini)* and capsule borer (*Helicoverpa armigera)* and their impact of seed yield Uttar Pradesh. *J. Zool*. pp: 291-294.

Patel RK and BS Thakur (2000) Insect pest complex and seasonal incidence in linseed with particular reference to bud fly (*Dasyneura lini* Barnes). *J. Plant Prot. Environ.* **2(2)**: 102-107.

Prasad R and D Prasad (2012) Seed yield loss in linseed due to bud fly (*Dasyneura lini* Barnes) under Ranchi conditions. *J. Eco-friendly Agric.* **7(1)**: 43-44.

Pal RK and R Singh (2010) Screening of linseed germplasm against bud fly *Dasyneura lini* Barnes. *Int. J. Plant Prot.* **3(2)**: 410-411.

Pradhan V, GP Painkra, KL Painkra and PK Bhagat (2018) Seasonal incidence of major insect pests in linseed. *J. Plant Dev. Sci.* **10(10)**: 579-581.

Pal R and YP Malik (2018) Screening of linseed germplasm against bud fly (*Dasyneura lini* Barnes) under field conditions. *Int. J. Pure Appl. Biosci*. **6(3)**: 196-201.

Pal R and YP Malik (2020) Histological basis of resistance in linseed against bud fly (*Dasyneura lini* Barnes) in Central Uttar Pradesh. *Int. Jour. Agric. Inv.* **5(1)**: 25-30.

Reddy MP, BR Reddy and JJ Maheshwari (2013) Screening of linseed genotypes for resistance against bud fly, *Alternaria* and Powdery mildew, genetic parameters for yield components in linseed (*Linum usitatissimum* L.). *Int. J. Curr. Microbiol. Appl. Sci.* ***2*(9)**: 267-276.

Rabindra P, A Kandulna and D Prasad (2005) Efficacy and economics of insect pest management in linseed grown under farmers and improved agronomical practices. *J. Plant Prot. Environ.* **2(2)**: 49-54.

Rabindra Prasad and Devendra Prasad (2012) Association between intensity of bud fly (*Dasyneura lini* Barnes) incidence and linseed seed yield. *Eco-friendly Agric. J.* **7(1)**: 49-50.

Sankari HS (2000) Linseed (*Linum usitatissimum* L.) cultivars and breeding lines as stem biomass producers. *J. Agron. Crop Sci.* **184(4)**: 225-231.

Singh PK and Ramanand (2000) Screening of linseed germplasm against bud fly infestation. *J. Crop Improv.* **27(2)**: 240-242.

Singh P, Sumit and DK Yadav (2015) Inheritance of resistance to bud fly infestation in linseed (*Linum usitatissimum* L.). *Indian J. Genet*. **75(3)**: 386-388.

Tripathi R, S Ali and R Kumar (2002) Screening of linseed germplasm against bud fly, (*Dasyneura lini* Barnes). *Dept. Ento.* **9(1)**: 85-86.

Tripathi R, S Ali and R Kumar (2003) Determination of economic threshold level of bud fly (*Dasyneura lini* Barnes) of linseed. *Shashpa* **10(2)**: 139 –141.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.07

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Elucidating Diversity among Mango (*Mangifera indica* L.) Hybrids based on Morphological Characters using DUS Guidelines

Gulshan Kumar[1], Manish Srivastav[1*], Sreekanth H.S.[1], Jai Prakash[1], Sanjay K. Singh[1] and Vinod[2]

Abstract

Mango (*Mangifera indica* L.) is adored by people owing to its delicious taste, aroma, sugar acid blend, attractive fruit and nutritional value. In the present investigation, genetic diversity among 24 mango hybrids bred at ICAR- Indian Agricultural Research Institute was observed using 11 quantitative and 16 qualitative traits as per the DUS guidelines on mango. Hybrids showed significant differences for leaf, inflorescence, panicle and fruit attributes. The maximum leaf area was recorded in H-4-8 (101.99 cm^2) and only two categories of leaf blade shapes were observed among hybrids. Most of the hybrids (91.6%) showed the presence of twisting leaf blade except H-3-2 and H-4-8. Pusa Manohari had the maximum anthocyanin coloration of rachis. Pusa Pratibha bloomed earliest, whereas Pusa Lalima showed earliest fruit maturity among hybrids. The maximum fruit weight and length (336.44 g, 14.56 cm) were observed in Mallika. Petiole length, leaf area, leaf blade length, leaf blade width and inflorescence width were recorded as highly diverse traits among mango hybrids. Results indicated that four superior hybrids, *viz*., H-4-8, NH-17-4, NH-18-4 and Pusa Arunima depicted strong peel chrominance which was majorly associated with export markets and consumer acceptance. The UPGMA dendrogram based on K–clustering grouped mango hybrids in to two major clusters. DUS fingerprint was generated using 16 qualitative traits. These would be helpful in the identification of these hybrids precisely. The information generated in the present study has significance in the conservation, cultivar improvement, protection and utilization of mango hybrids in future.

Keywords: Clustering, DUS guidelines, Fingerprint, Hybrids, *Mangifera indica*, Mango.

[1]Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India

[2]Division of Genetics, ICAR- Indian Agricultural Research Institute, New Delhi, India

***Author for correspondence:**

mns_fht@rediffmail.com

Received: 01/01/2023 **Revised:** 02/02/2023

Accepted: 06/06/2023

How to cite this article: Kumar, G., Srivastav, M., Sreekanth, H.S., Prakash, J., Singh, S.K., Vinod. (2023). Elucidating Diversity among Mango (*Mangifera indica* L.) Hybrids based on Morphological Characters using DUS Guidelines. Indian J. Plant Genetic Resources. 36(3), 387-395. **DOI:** 10.61949/0976-1926.2023.v36i03.07

Introduction

Mango (*Mangifera indica* L.) a member of the family Anacardiaceae, is acclaimed as national fruit crop in India, and is grown in a wide range of tropical and sub-tropical climates all over the world. Mango production in the world is projected to exceed 55.38 million MT, and India as the top mango producer in the world produces 20.44 million MT of mangoes from an area of 2.29 million ha (NHB, 2019-20). India offers a huge export potential for this fruit and exported about 27,872.06 MT of fresh mango valuing 327.42 crore (APEDA, 2021-22). Though India is the largest producer of the choicest varieties of mango, its share in the international market is abysmally low. In India, there are over a thousand different mango varieties (Mukherjee, 1951). Despite having so much diversity for this fruit crop in our country, only a dozen are under commercial cultivation. Most of these varieties have one or other defects like poor shelf-life, spongy tissue, mango malformation and alternate bearing.

In India, mango improvement work was initiated in the year 1911 at Pune, Maharashtra (Burns and Prayag, 1921) but has not been pursued after to yield any useful information. Later, at the ICAR-Indian Agricultural Research Institute (ICAR-IARI), New

Delhi, systematic hybridization work was started in 1961, which resulted in the development of two regular bearing popular hybrids, namely, Mallika (Neelum x Dashehari) and Amrapali (Dashehari x Neelum) (Singh *et al.,* 1972). The emphasis later shifted towards developing red peel-colored mango cultivars targeting overseas markets. This led to the development and release of a series of improved mango cultivars with red peel and good fruit quality, namely, Pusa Arunima in 2002, and Pusa Pratibha, Pusa Shrestha, Pusa Peetamber and Pusa Lalima hybrid varieties in 2012, which have been released by central variety release committee for commercial cultivation at national level in 2021. In 2021, two more mango hybrids, Pusa Deepshikha and Pusa Manohari, were released by the Delhi State Seed Sub-committee for commercial cultivation in the National Capital Region. At ICAR-IARI, New Delhi mango hybridization work is in progress and several hundred hybrids obtained from different cross combinations have been evaluated. Characterization of these hybrids using DUS guidelines is of utmost importance to safeguard the protection of these valuable mango hybrids. DUS Testing is the standard process of evaluating plant varieties for distinctiveness, uniformity, and stability. Bhamini *et al.* (2018) illustrated the significance of DUS Descriptor developed by PPV & FR, 2001 and marked its esteemed importance in diversity analysis studies. The stable plant characteristics that are unaffected by the environment are often taken into consideration during DUS characterization. The present study aimed to characterize mango hybrids bred at ICAR- IARI, New Delhi based on DUS guidelines, which would help in varietal identification, protection and resolve intellectual property rights issues. The information gained in the present investigation will also be useful for selecting desirable traits to develop superior hybrids.

Material and Methods

Plant Material

In 24 mango hybrids, bred at the ICAR- IARI, New Delhi aged 10 to 20 years were selected for the studies (Table 1). These mango hybrids have been maintained under uniform cultural practices during the period of investigation.

Morphological Analysis

Mango hybrids were characterized for leaf, inflorescence and fruit parameters based on standard DUS guidelines on mango (PPV & FRA)-2020 and Mango Descriptor, IPGRI, Rome Italy. Twenty- seven (11 quantitative and 16 qualitative) traits representing leaf, inflorescence and fruits were studied over two years. The experiment followed a randomized block design. The morphological traits were assessed using 10 replications from relevant parts taken from each plant. Mature leaves were taken from the middle third portion of young shoot. The leaf blade length, width, and area were measured with WinFOLIA™ software using leaf area meter (*REGENT*, Canada). Petiole length was measured using a digital Vernier Caliper (Mitutoya Model 500-147). Mango hybrids were categorized into early, medium and late groups based on the time of 50% flowering on the tree. The inflorescence length and inflorescence width were measured using a measuring scale. Fruits were harvested at mature firm stage based on standard maturity criterion and ripened at ambient temperature. Fruit length and width was measured using a digital Vernier calliper. Fruit weight was measured with the help of digital electronic balance (Adiar Dutt-1620C, USA). Intensity of anthocyanin pigmentation, leaf blade shape, leaf color, twisting of leaf blade, shape of leaf base, the shape of leaf apex, anthocyanin coloration of rachis, shape of mature fruit, color of mature fruit, presence of cavity at stalk, fruit beak type, sinus type, depth of sinus and maturity of fruits were observed based on categories given in mango DUS guidelines and descriptor.

Cluster Analysis and DUS Fingerprinting

The UPGMA clustering and principal component analysis (PCA) was carried out using IBM SPSS Statistics ver. 26 software. A fingerprint of 24 mango hybrids based on horticultural traits mentioned in DUS guidelines has also been generated using Microsoft Excel 2019.

Results

Characterization Based on Quantitative Traits

In the present investigation, significant variations were observed for the majority of quantitative traits. Leaf blade length ranged from 14.87 cm (Pusa Pratibha) to 24.70 cm (H-4-8), and leaf blade width varied from 3.87 cm (NH-19-2) to 5.83 cm (H-4-8). Significant variation was also evident for petiole length, and mango hybrids grouped into two categories based on medium and long petiole types (Table 2). Leaf area ranged from 45.99 to 101.99 cm^2, and significant varied among hybrids. The maximum (101.99 cm^2) leaf area was in H-4-8 and the minimum (45.99 cm^2) was in Pusa Pratibha. The data showed significantly variation for inflorescence length and it ranged from 18.11 to 46.58 cm among hybrids. The maximum inflorescence length (46.58 cm) was observed in H-7-1 followed by Pusa Lalima (41.91 cm) and H-3-2 (37.42 cm), while the minimum was recorded in NH-20-2 (18.11 cm), NH-17-3 (19.51 cm) and Pusa Peetamber (22.56 cm), respectively. Similarly, inflorescence width among hybrids varied from 8.50 to 12.42 cm. The minimum inflorescence width was noted in H-12-5 (8.50 cm) followed by NH-20-2 (8.85 cm) and H-4-8 (8.91 cm). A significant level of variation was also noted for the fruit length among mango hybrids. The maximum fruit length was noted in Mallika (14.56 cm) while it was minimum (7.56 cm) in NH-17-3. The fruit width was maximum (7.60 cm) in Mallika and minimum (4.38 cm)

Table 1: List of mango (*Mangifera indica* L.) hybrids used in the present study

Sr. No.	*Hybrid*		*Parentage*
1	Amrapali	:	Dashehari x Neelum
2	H-1-11	:	Amrapali x Sensation
3	H-12-5	:	Amrapali x Sensation
4	H-1-5	:	Amrapali x Sensation
5	H-2-14	:	Amrapali x Alphanso
6	H-3-2	:	Amrapali x Sensation
7	H-4-8	:	Amrapali x Sensation
8	H-7-1	:	Amrapali x Sensation
9	Mallika	:	Neelum x Dashehari
10	NH-16-2	:	Amrapali x Sensation
11	NH-17-1	:	Amrapali x Sensation
12	NH-17-3	:	Amrapali x Sensation
13	NH-17-4	:	Amrapali x Sensation
14	NH-18-4	:	Amrapali x Sensation
15	NH-19-2	:	Amrapali x Sensation
16	NH-19-3	:	Amrapali x Sensation
17	NH-20-2	:	Amrapali x Sensation
18	Pusa Arunima	:	Amrapali x Sensation
19	Pusa Deepshikha	:	Amrapali x Sensation
20	Pusa Lalima	:	Dashehari x Sensation
21	Pusa Manohari	:	Amrapali x Lal Sundari
22	Pusa Peetamber	:	Amrapali x Lal Sundari
23	Pusa Pratibha	:	Amrapali x Sensation
24	Pusa Shreshth	:	Amrapali x Sensation

H-hybrid, NH- New hybrids

in NH-17-4. Among hybrids, fruit shape index ranged from 1.40 to 2.33, indicating oblong to roundish fruits. A perusal of data indicates that there was a wide range of variation for fruit weight among mango hybrids and varied from 94.87 to 336.44 g. The maximum fruit weight (336.44 g) was recorded in Mallika, followed by Pusa Deepshikha (276.35 g) while the minimum fruit weight (94.87 g) was observed in NH-19-2 followed by NH-17-4 (99.31 g).

Morphological Characterization Based on Qualitative Traits

In the present study, 16 qualitative traits were included to characterize 24 mango hybrids. The distribution of each trait was found to be polymorphic among hybrids. Among 24 mango hybrids, only two categories of leaf blade shapes were observed (i) elliptic (96%) and (ii) oblong (4%). Most of the mango hybrids showed the presence of twisting leaf blade (91.6%). The majority of hybrids were observed with an acute (96%) type leaf base shape. However, NH-17-1 had an obtuse type of leaf base. Out of the 24 mango hybrids, 18 hybrids (75%) had an attenuate type of leaf apex shape, and the remaining 6 hybrids (25%) were observed with an acuminate type of leaf apex. Two types of leaf color, including light green (12%) and dark green (88%) were observed. Out of 24 hybrids, only three classes of leaf anthocyanin intensity were recorded, *i.e.*, weak (25%), medium (46%) and strong (29%). Flowering-related traits, including anthocyanin coloration of rachis and time of 50% flowering, were observed. It was evident that 45.8% of mango hybrids had medium type of coloration on rachis, and most of mango hybrids (50%) were recorded with weak or absence of anthocyanin coloration of rachis. Pusa Manohari was the only hybrid to have a strong anthocyanin coloration of rachis. Among 24 mango hybrids, Pusa Pratibha was found to have the earliest initiation of flowering. Observation on mature fruit color revealed that eight hybrids (33.33%) had green and yellow peel color, whereas seven hybrids (29.16%) had green and orange peel color, four hybrids (16.66%) had green and pink peel color, four hybrids (16.66%) with green and purple peel color and only one hybrid observed to have only green coloration for fruit peel, and highest diversity index (1.45) in mature fruit color trait was observed (Table 3). Most of the mango hybrids (71%) did not have a cavity at the fruit stalk, and only seven hybrids (29%) had shallow cavity at the fruit stalk. The prominence of fruit beak was observed as a significant attribute that influences customer acceptability and marketing of mango fruits. Out of 24 mango hybrids, 50% of hybrids had a perceptible beak. Whereas 12.50% of hybrids registered a pointed type of beak and 37.50% of hybrids were observed with a prominent type of beak. Noticeable variations for the fruit sinus were also observed and 70.83% of hybrids had sinuses and 29.16% of the hybrids without sinus. The majority of hybrids (70.83%) had shallow sinuses and (29.16%) of hybrids had no sinuses. Overall, 66.66% of hybrids had oblong, 16.66% were obovoid, 8.33% roundish and 8.33% with ovoid fruit shapes were observed. Results pertaining to fruit maturity indicated that the earliest fruit maturity was observed only in Pusa Lalima and 11 hybrids had medium maturity. In contrast, 12 hybrids were found to be of late maturity group.

Cluster Analysis Based on Morphological Traits

Cluster analysis of mango hybrids was carried out based on the observations on morphological parameters studied. Two-step hierarchical clustering and K-mean cluster analysis were used using IBM SPSS Statistics ver. 26 software. K–clustering offered more stable results when compared to hierarchical clustering. Cluster membership indicated that 24 hybrids were categorized into two major clusters. Cluster I included maximum number of mango hybrids (17) comprising Amrapali, H-12-5, H-1-5, H-2-14, Mallika, NH-16-2, NH-17-1, NH-17-3, NH-17-4, NH-19-2, NH-19-3, NH-20-2,

Table 2: Morphological characterization of different mango hybrids based on DUS guidelines

Hybrid	*Leaf blade length*	*Leaf blade width*	*Petiole length*	*Leaf blade shape*	*Twisting of leaf blade*	*Shape of leaf base*	*Shape of leaf apex*	*Leaf colour*	*Leaf anthocyanin colouration*	*Inflore-scence length*	*Inflore-scence width*
Amrapali	5	5	5	5	9	3	5	7	5	5	7
H-1-11	5	5	7	5	9	3	3	7	5	7	7
H-12-5	5	5	5	5	9	3	5	3	7	5	5
H-1-5	5	5	5	5	9	3	3	7	5	5	5
H-2-14	5	5	7	5	9	3	3	7	3	7	7
H-3-2	7	5	5	7	1	3	5	7	5	7	7
H-4-8	7	5	5	5	1	3	3	7	5	5	5
H-7-1	5	5	5	5	9	3	3	7	3	7	7
Mallika	5	5	5	5	9	3	3	3	5	7	7
NH-16-2	5	5	5	5	9	3	3	7	3	7	7
NH-17-1	5	5	7	5	9	5	5	3	7	7	5
NH-17-3	5	5	7	5	9	3	3	7	3	3	7
NH-17-4	5	5	5	5	9	3	3	7	5	7	7
NH-18-4	5	5	5	5	9	3	5	7	3	5	5
NH-19-2	5	5	7	5	9	3	3	7	5	5	5
NH-19-3	5	5	7	5	9	3	3	7	7	5	7
NH-20-2	5	5	7	5	9	3	3	7	7	3	5
Pusa Arunima	5	5	5	5	9	3	3	7	5	5	7
Pusa Deepshikha	5	5	5	5	9	3	3	7	7	7	7
Pusa Lalima	5	5	5	5	9	3	3	7	5	7	7
Pusa Manohari	5	5	7	5	9	3	5	7	5	5	5
Pusa Peetamber	7	5	7	5	9	3	3	7	7	5	7
Pusa Pratibha	5	5	5	5	9	3	3	7	7	5	7
Pusa Shreshth	5	5	5	5	9	3	3	7	3	7	7

Leaf blade length (3- short (<12 cm), 5- medium (12-22 cm) and long (>22 cm); Leaf blade width (3- narrow (<3 cm), 5- medium (3-6 cm) and 7-broad (>6 cm); Petiole length (3- short (<1.5 cm), 5- medium (1.5-3.0 cm) and 7- long (>3 cm; Leaf blade shape (3-ovate, 5- elliptic, 7- oblong); Twisting of leaf blade (1- absent, 9- Present); Shape of leaf base (3- acute, 5- obtuse, 7- rounded); Shape of leaf apex (3- attenuate, 5- acuminate, 7- acute); Leaf colour (3- light green, 7- dark green);Young leaf: intensity of leaf anthocyanin (1- absent, 3- weak, 5- medium, 7- strong); Inflorescence length (3- short (<20 cm), 5- medium (20-30 cm) and 7- long (>30 cm); Inflorescence width (3- short (<7.5 cm), 5- medium (7.5-15 cm) and 7- long (>15 cm)

Pusa Arunima, Pusa Lalima, Pusa Manohari, Pusa Pratibha and Pusa Shreshth while, cluster II comprised of remaining seven hybrids, viz., H-1-11, H-3-2, H-4-8, H-7-1, NH-18-4, Pusa Deepshikha and Pusa Peetamber (Figure 1). ANOVA based on K–clustering showed that out of 23 traits, 5 traits *viz*. twisting of leaf blade, fruit sinus type, depth of sinus, mature fruit shape and leaf blade length were found to be significant across the two clusters.

DUS Fingerprints of Mango Hybrids

DUS fingerprints of mango hybrids have been considered important horticultural traits for the conduct of test for distinctiveness, uniformity and stability on mango and presented in Table 4. The fingerprints include 14 morphological informative traits representing 24 mango hybrids. Different color codes were given for traits showing variation for each character and similar kind of pattern was also followed in 24 mango hybrids. Identification of these mango hybrids at particular crop growth phases could be made easier using these DUS fingerprints. It was found that three characters, *viz*., leaf blade shape, shape of leaf base and time of 50% flowering, were highly informative among all characters for the mango hybrids, namely, H-3-2, NH-17-1 and Pusa Pratibha.

Discussion

The present demand of trait-specific varieties has resulted in a shift of mango breeding objectives to develop more

Table 2. Cont: Morphological characterization of different mango hybrids based on DUS guidelines

Hybrid	*Anthocyanin colouration of rachis*	*Time of flowering: 50% of tree*	*Fruit length*	*Fruit width*	*Mature fruit colour*	*Presence of cavity at stalk*	*Depth of cavity at stalk*	*Fruit sinus type*	*Depth of sinus*	*Fruit beak type*	*Mature fruit shape*	*Maturity group: Fruits ready to harvest*
Amrapali	3	5	5	5	3	1	1	1	1	1	1	7
H-1-11	1	5	7	5	3	9	3	9	3	3	5	5
H-12-5	1	5	5	5	5	1	1	1	1	1	5	5
H-1-5	3	5	7	5	3	1	1	9	3	1	1	7
H-2-14	3	5	5	5	5	1	1	1	1	1	1	5
H-3-2	3	5	7	5	3	9	3	9	3	2	5	7
H-4-8	1	5	5	5	9	9	3	9	3	3	1	7
H-7-1	1	5	5	5	3	9	3	9	3	3	2	5
Mallika	1	5	7	7	5	1	1	9	3	2	1	5
NH-16-2	1	5	5	5	1	1	1	9	3	1	1	5
NH-17-1	1	5	5	3	3	1	1	1	1	1	1	7
NH-17-3	1	5	5	5	3	1	1	9	3	1	1	7
NH-17-4	1	5	7	3	9	1	1	9	3	3	1	7
NH-18-4	3	5	5	5	9	9	3	9	3	2	5	7
NH-19-2	3	5	7	3	5	1	1	9	3	3	1	5
NH-19-3	3	5	5	5	3	1	1	9	3	3	1	7
NH-20-2	3	5	5	5	5	1	1	9	3	3	3	7
Pusa Arunima	3	5	7	5	9	1	1	1	1	1	1	7
Pusa Deepshikha	1	5	7	5	7	9	3	9	3	3	2	7
Pusa Lalima	1	5	7	5	7	1	1	9	3	1	1	3
Pusa Manohari	5	5	7	5	5	1	1	9	3	3	1	5
Pusa Peetamber	3	5	5	5	5	9	3	9	3	1	3	5
Pusa Pratibha	3	3	7	7	7	1	1	1	1	1	1	5
Pusa Shreshth	1	5	7	5	7	1	1	1	1	1	1	5

Inflorescence colouration : Anthocyanin coloration of rachis(1- absent or weak, 3- medium, strong- 5); Time of flowering 50% of the tree(3- early, 5- medium, 7- late); Fruit length (3- short (<5 cm), 5- medium (5-10 cm), 7- long (10-20 cm) and 9- extra long (>20 cm) ; Fruit width 3- (narrow (<5 cm), 5- medium (5-7 cm) and 7- broad (>7 cm); Mature fruit colour(1- only green, 3- green and yellow, 5- green and orange, 7- green and pink, 9- green and purple); Presence of cavity at stalk(1- absent, 9- absent); Depth of cavity at stalk (1- absent, 3- shallow); Fruit sinus type (1- absent, 9- present); Depth of Sinus (1- absent, 3- shallow); Fruit beak type (1- perceptible, 2- pointed, 3- prominent, 4- mammiform); Mature fruit shape (1- oblong, 2- elliptic, 3- roundish, 4- ovoid, 5- obovoid, 99- other); Maturity group: Fruits ready to harvest (3- early, 5- medium, 7- late

specific and consumer-oriented varieties. Horticultural traits, *viz*., red peel color, moderate sweetness, medium to large size fruits, dwarfness, salt tolerance, climate resilience, and resistance against emerging pests and diseases, are important in mango improvement. ICAR- Indian Agricultural Research Institute had stepped forward with the same objectives and released several new promising mango hybrids. Characterization of newly bred hybrids is of utmost importance for registration and protection of these mango hybrid varieties. DUS guidelines are one such approach to assist this and are widely utilized in many fruit crops (Krishna *et al.,* 2016; Wani *et al.,* 2017; Sridhar and Babu, 2017; Bhamini *et al.,* 2018; Dinesh *et al.,* 2018; Molla *et al.,* 2019; Jena *et al.,* 2021; Wang *et al.,* 2022 and Rasool *et al.,* 2022). DUS traits given in guidelines for the conduct of test for distinctiveness, uniformity and stability on mango are considered stable traits and less affected by environmental conditions. In the present study, mango hybrids showed significant variations for leaf length, width and area. It is indeed possible that cultivar differences arise due to the involvement of different heterozygous parents and thus can be attributed to variation in leaf size. Dinesh *et al.* (2018) also observed significant variability in leaf characters based on DUS guidelines among mango hybrids. Considering that

Table 3: Frequency distribution of quantitative and qualitative traits among 24 mango hybrids

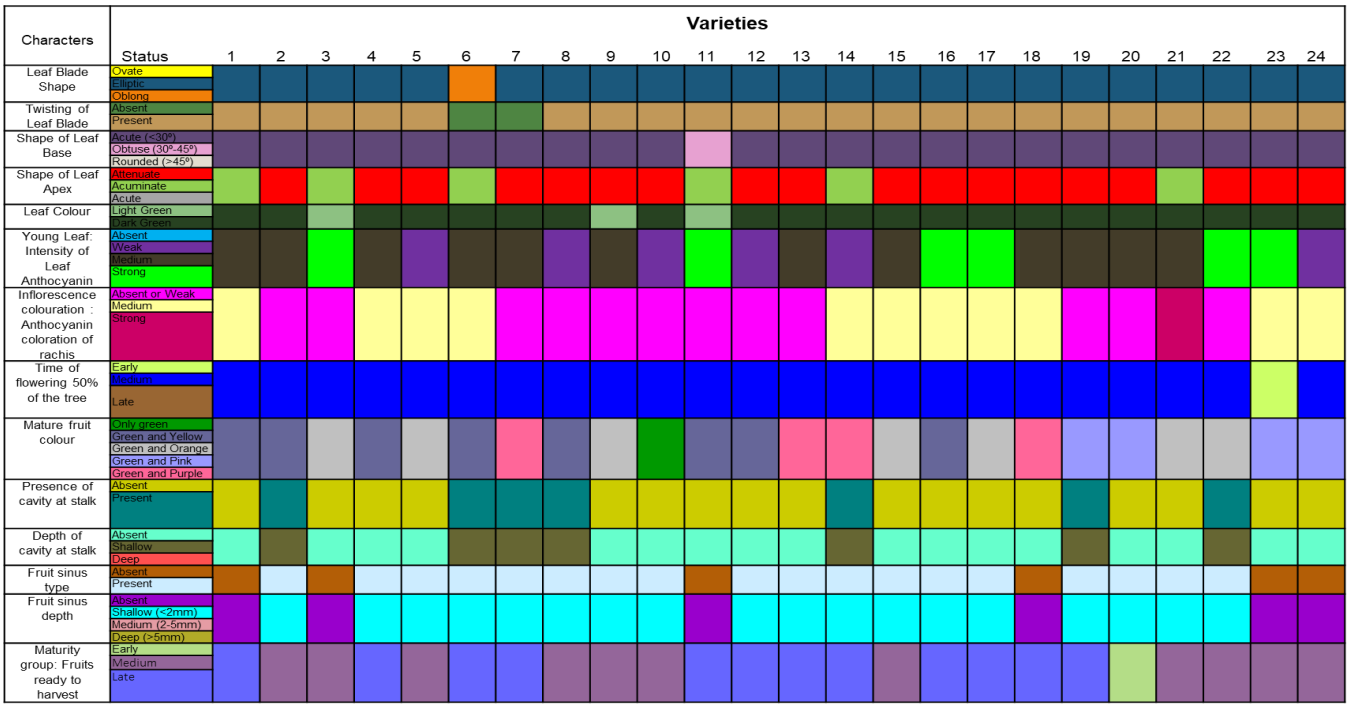
Trait	*Scale*	*F*	*RF(%)*	*DI*	*Trait*	*Scale*	*F*	*RF(%)*	*DI*
Leaf blade length	Long	3	12.50	0.37	Time of flowering: 50% of the tree	Early	1	4.16	0.17
	Medium	21	87.50			medium	23	95.83	
Leaf blade width	Medium	24	100	0	Fruit length	Medium	12	50	0.69
Petiole length	Long	9	37.50	0.66		Long	12	50	
	Medium	15	62.50		Fruit width	Broad	2	8.33	0.65
Leaf blade shape	Oblong	1	4.16	0.17		Narrow	3	12.50	
	Elliptic	23	95.83			Medium	19	79.16	
Twisting of leaf blade	Absent	2	8.33	0.28	Mature fruit colour	Only green	1	4.16	1.45
	Present	22	91.66			Green and purple	4	16.66	
Shape of leaf base	Obtuse	1	4.16	0.17		Green and pink	4	16.66	
	Acute	23	95.83			Green and orange	7	29.16	
Shape of leaf apex	Acuminate	6	25	0.56		Green and yellow	8	33.33	
	Attenuate	18	75		Presence of cavity at stalk	Present	7	29.16	0.60
	Acute	17	58.62			Absent	17	70.83	
Leaf colour	Light green	3	12.50	0.37	Depth of cavity at stalk	Shallow	7	29.16	0.60
	Dark green	21	87.50			Absent	17	70.83	
Intensity of leaf anthocyanin	Weak	6	25.00	1.06	Fruit sinus type	Absent	7	29.16	0.60
	Strong	7	29.16			Present	17	70.83	
	Medium	11	45.83		Depth of sinus	Absent	7	29.16	0.60
Inflorescence length	Short	2	8.33	0.92		Shallow	17	70.83	
	Medium	11	45.83		Fruit beak type	Pointed	3	12.5	0.97
	Long	11	45.83			Prominent	9	37.5	
Inflorescence width	Medium	8	33.33	0.63		Perceptible	12	50	
	Long	16	66.66		Mature fruit shape	Roundish	2	8.33	0.98
Anthocyanin coloration of rachis	Strong	1	4.13	0.83		Elliptic Obovoid	2 4	8.33 16.66	
	Medium	11	45.83			Oblong	16	66.66	
	Absent or weak	12	50.00						
					Maturity groups: Fruit ready to harvest	Early Medium Late	1 11 12	4.16 45.83 50.00	0.83

F- Frequency, RF- Relative frequency and DI – Diversity index

plants with greater leaf areas offer higher photosynthetic rates and may be affecting the fruit size and quality. The present findings corroborated with the findings of Rhodes *et al.* (1970); Rajwana *et al.* (2011); Joshi *et al.* (2013); Wani *et al.* (2017) and Balamohan and Vidhya (2020).

Leaf qualitative traits among mango hybrids also differed significantly and in conformity to the similar findings by Jena *et al.* (2021) who reported oblong leaf shape in most of the genotypes and ranging from rounded to acuminate. In the present studies, the acuminate type of leaf apex was

Table 4: DUS fingerprints of mango hybrids based on horticultural traits



1- Amrapali, 2- H-1-11, 3- H-12-5, 4- H-1-5, 5- H-2-14 , 6- H-3-2 , 7- H-4-8, 8- H-7-1, 9- Mallika, 10- NH-16-2, 11- NH-17-1, 12- NH-17-3, 13- NH-17-4. 14- NH-18-4, 15- NH-19-2, 16- NH-19-3, 17- NH-20-2, 18- Pusa Arunima, 19- Pusa Deepshikha, 20- Pusa Lalima, 21- Pusa Manohari, 22- Pusa Peetamber, 23- Pusa Pratibha, 24- Pusa Shreshth

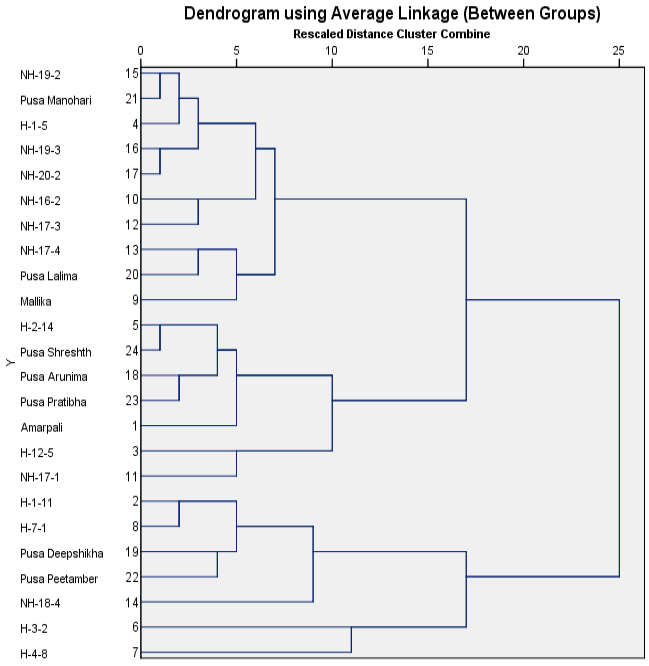


Figure 1: Dendrogram depicting average linkage between mango hybrids

recorded in hybrids, namely, Amrapali, H-12-5, H-3-2, NH-17-1, NH-18-4 and Pusa Manohari. Likewise, mature leaf color results were in accordance with the findings of Fivaz (2008). Results pertaining to intensity of anthocyanin coloration in young leaves clearly indicated significant variation in three distinct classes: weak, medium and strong. Earlier, Molla *et al.* (2019) also reported such findings in mango genotypes of Bangladesh. Mango leaves can change color as they mature, transitioning from copper brown to light greenish and dark green as reported by Mezel *et al.* (2017). As the juvenile leaves grows they tend to accumulate more chlorophyll and their net carbon absorption rises initially from net carbon importers to net CO_2 assimilation.

Every mango genotype has a distinct flowering period thus it may be often tied to the prevailing environmental conditions, genetics, nutrition levels, and hormonal elements (Kulkarni, 2004). Present findings are in agreement with these reports and showed that mango hybrids had different flowering times. Pusa Pratibha was recorded with the earliest flowering amongst all hybrids during the period of study. Inflorescence length and width are key factors influencing the pollinator activity and fruit set by offering a larger surface area. We observed the maximum inflorescence length (46.58 cm) in H-7-1. Pandit *et al.* (2017) also reported similar kind of findings based on DUS characterization.

Twelve hybrids (50%) had fruit lengths more than 10 cm. Ranjith *et al.* (1982) also hypothesized that environmental influences may have a greater impact on fruit size than genetics alone. The fruit size is highly dependent on initial cell division and further enlargement of these cells. Prevailing environmental condition significantly influence division and enlargement of cell. Using its resources effectively, a cultivar may have the innate potential to yield fruits of greater size. Kumar *et al.* (2016) also reported similar

results pertaining in apples under the northern Himalayas. It has been suggested that mango fruit shape has a significant role in packaging and transportation. Our results showed considerable variation in fruit shape index. Amongst mango hybrids, NH-17-1 had the maximum (2.33) fruit shape index at par with NH-19-2 (2.29) and NH-17-1 (1.93), suggesting their elliptical or oblong fruit shape. The data regarding fruit weight of different mango hybrids suggested a wide range of variations ranging from 94.87 to 336.44 g. Medium-sized mango fruits between 200 to 250 g are preferred for marketing. Mallika had the maximum (336.44 g) fruit weight while the minimum (94.87 g) was in NH-19-2. Similar variations have also been noticed in mango by Wani *et al.* (2017); Molla *et al.* (2019) and Mango Genome Consortium *et al.* (2021). Our findings strongly agree with the findings of Rymbai *et al*. (2014), who speculated that transgressive segregation or additive gene action might impact the quantitative characteristic in mango hybrids. One of the key fruit quality attributes that determine the attractiveness and marketability of the fruits is their aesthetic and visual appeal. Fruit peel color was an incredibly variable trait spread over 5 phenotypic classes. Thus, a useful color library for identifying distinct mangoes may be created. Singh *et al.* (2012) also reported a wide range of peel coloration amongst mango hybrids and further suggested their utilization in developing red peel color hybrids which fetch more price in international markets. In the present study, a positive correlation was observed between the presence of cavities and depth at the stalk. It was noted that amongst 24 hybrids, a shallow cavity was present in 70.83%. A similar kind of correlation exists between fruit sinus and depth of sinus. Additionally, mangoes are categorized in the global market according to customer preferences for shape and peel color (Campbell, 1995). The maximum (66.66%) hybrids in the present investigation had an oblong fruit shape. Shamili *et al.* (2012) evaluated fruit morphologies and found that Iranian genotypes exclusively produced elongated and oblong fruit shapes. It was interesting to note that all the hybrids can be broadly grouped into 3 groups for fruit maturity. Pusa Lalima has been documented with early fruit maturity. Additionally, it has been discovered that elevated and fluctuating temperatures during the early stages of fruit growth have an impact on fruit maturity (Kumar *et al.,* 2016).

Significant variations between mango hybrids were observed in terms of both qualitative and quantitative traits. Out of 23 traits, 5 were significant ($p \leq 0.001$) across the two clusters. Earlier, Harisha *et al.* (2021) developed a similar kind of DUS fingerprint while studying diversity analysis in rice genotypes and reported its efficiency in the identification of informative traits. In the present investigation, morphological parameters suggested in DUS guidelines have been used for generation of fingerprints for mango hybrids. This fingerprint is highly useful in the future identification, protection and conservation of mango.

Conclusion

Twenty-four mango hybrids have been characterized based on DUS guidelines and hybrid-specific stable morphological traits have been identified. The unique traits have immense value in identifying, protecting and conserving these hybrids. Furthermore, the genetic relatedness based on the diversity analysis provided useful information for the utilization of these hybrids in future breeding programmes as the source of important desirable traits. Attractive fruit peel color, early fruit maturity, and fruit weight are traits of economic importance that must be utilized for mango improvement in future breeding programs. DNA fingerprints based on DUS guidelines will further help to identify and protect these hybrids.

Acknowledgments

The first author is thankful to the Indian Council of Agricultural Research (ICAR), New Delhi for the kind Junior Research Fellowship (JRF) grant for the master's degree programme. The authors are thankful to ICAR- IARI, New Delhi, for providing facilities to carry out the essential research work.

References

APEDA (2020-21). Agricultural and Processed Food Products Export Development Authority. *India Exports Statistics*. New Delhi, India. *https://agriexchange.apeda.gov.in/*

Balamohan, T. N., & Vidhya, D. (2020). Evaluation of traditional mango (Mangifera indica L.) varieties of Tamil Nadu for fruit quality attributes. *Int. j. chem. biochem. sci.*, **18**: 57-62.

Bhamini, K., Jaiswal, U. S., Ahmad, M. F., & Nayyer, A. (2018). Studies on the physio-morphology, flowering and fruit characteristics of mango genotypes in subtropical climate of Eastern India. *J. pharmacogn. phytochem.*, **7(2)**: 3783-3789.

Burns, W., & Prayag, S. H. (1921). The book of the mango. Department of Agriculture, Bombay. *Bull*, *103*.

Campbell, C. D. B., & Bernard, R. (1995). Mangos in the United States: A year long supply. In *Florida State Horticultural Society. Meeting (USA)*, **107***:* 303-334.

Menzel, C. M., & Le Lagadec, M. D. (2017). Can the productivity of mango orchards be increased by using high-density plantings? *Sci. Hortic.*, **219**: 222-263.

Dinesh, M. R., Ravishankar, K. V., Gowda, D. S., & Sankaran, M. (2018). Morphological characterization and genetic barcoding of kuttiatoor mango accessions.

Fivaz, J. (2008). Botanical aspects. In: de Villiers EA, Joubert PH (Eds.) The Cultivation of Mango. ARC Institute for Tropical and Subtropical Crops, Florida, USA, pp 9-20.

Harisha, R., Bhadru, D., Vanisri, S., Gourishanakar, V., & Satish, L. (2021). SSR and morphological traits based fingerprints and DNA barcodes for varietal identification in rice. *Biotechnol. Biotechnol. Equip.*, **35(1)**: 1461-1473.

Jena, R. C., Agarwal, K., & Chand, P. K. (2021). Fruit and leaf diversity of selected Indian mangoes (*Mangifera indica* L.). *Sci. Hortic.*, **282**: 109941.

Joshi, R., Kundu, M., & Singh, C. P. (2013). Morphological characters: efficient tool for identification on different mango cultivars.

Environ. Ecol., **31(1A)**: 385-388.

Krishna, H., Bhargava, R., Chauhan, N., & Sharma, S. K. (2016, June). Morphological descriptor for DUS testing of Indian jujube (*Ziziphus mauritiana*). *Indian J. Agric. Sci.,* **86(6)**: 809-814.

Kulkarni, V. J. (2004). The tri-factor hypothesis of flowering in mango. In *VII International Mango Symposium 645* (pp. 61-70).

Kumar, J., Thakur, D., & Thakur, M. (2016). Long Term Performance of Some Spurs and Colour Strains of Apple (*Malus domestica* Borkh.) under Low Altitude Conditions of Kullu Valley of Himachal Pradesh. *Int. j. bio-resour. stress manag.*, **7(1)** 40-46.

Mango Genome Consortium, Bally, I. S., Bombarely, A., Chambers, A. H., Cohen, Y., Dillon, N. L., ... & Yan, H. (2021). The 'Tommy Atkins' mango genome reveals candidate genes for fruit quality. *BMC Plant Biology*, **21**: 1-18.

Molla, M. R., Ahmed, I., Hossain, M. A., Islam, M. S., Chowdhury, M. A. Z., Shabnam, D., & Rohman, M. M. (2019). Morphological characterization and Simple Sequence Repeats (SSRs) based DNA fingerprinting of selected mango (*Mangifera indica* L.) genotypes in Bangladesh. *j. hortic. for.*, **11(7)**: 104-119.

Mukherjee, S. K. (1951). The origin of mango. *Indian J Genet Plant Breed., 2*, 49.

NHB (2019-20). *Indian Horticulture Database*. Gurugram, India. *http://www.nhb.com*

Pandit, B. A., Wani, M. S., Dar, S. A., & Rashid, R. (2017). Blooming behavior of exotic apple cultivars under North Himalayan Region of India. *Int J Curr Microbiol Appl Sci, Int J Curr.*, **6(2)**: 1765-1775.

PPV & FRA (2020). Guidelines for conduct of test for DUS Mango (*Mangifera indica* L.), Protection of Plant Varieties and Farmers Right Authority, GOI, NASC Complex, DPS Marg, New Delhi, 17 P

Rajwana, I. A., Khan, I. A., Malik, A. U., Saleem, B. A., Khan, A. S., Ziaf, K., ... & Amin, M. (2011). Morphological and biochemical markers for varietal characterization and quality assessment of potential indigenous mango (*Mangifera indica*) germplasm. *International Journal of Agriculture and Biology*, **13**: 151-158.

Ranjith, P. J. (1982). Evaluation of mango *(Mangifera indica* L.) hybrids for certain useful characters. M.Sc. thesis submitted to the PG School IARI, New Delhi, 69 p.

Rasool, A., Bhat, K. M., Mir, M. A., Jan, A., Dar, N. A., & Mansoor, S. (2022). Elucidating genetic variability pertaining to flowering, maturity and morphological characters among various apple (*Malus domestica* Borkh.) cultivars. *S. Afr. J. Bot.*, **145**: 386-396.

Rhodes, A. M., Campbell, C., Malo, S. E., & Carmer, S. G. (1970). A Numerical Taxonomic Study of the Mango (*Mangifera indica* L.). *J. Am. Soc. Hortic. Sci.*, **95(2)**: 252-256.

Rymbai, H., Laxman, R. H., Dinesh, M. R., Sunoj, V. J., Ravishankar, K. V., & Jha, A. K. (2014). Diversity in leaf morphology and physiological characteristics among mango (*Mangifera indica* L.*)* cultivars popular in different agro-climatic regions of India. *Sci. Hortic.*, **176**: 189-193.

Shamili, M., Fatahi, R., & Hormaza, J. I. (2012). Characterization and evaluation of genetic diversity of Iranian mango (*Mangifera indica* L., Anacardiaceae) genotypes using microsatellites. *Sci. Hortic.*, **148**: 230-234.

Singh, A., Singh, A. K., & Singh, S. K. (2012). SSR markers reveal genetic diversity in closely related mango hybrids. *Indian J Hortic*, **69(3)**: 299-305.

Singh, R. N., Majumder, P. K., Sharma, D. K., & Mukherjee, S. K. (1972). Some promising mango hybrids. *Acta Hortic.,* **24**: 117-119.

Sridhar, R., & Harish Babu, B. N. (2017, September). A novel pomegranate (*Punica granatum* L.) variant with high anthocyanin content. In *International Symposium on Horticulture: Priorities and Emerging Trends,* **1255**: 149-152.

Wang, Y., Hu, G. P., Liu, Z. S., Zhang, J., Ma, L., Tian, T., ... & Wang, X. R. (2022). Phenotyping in flower and main fruit traits of Chinese cherry [*Cerasus pseudocerasus* (Lindl.) G. Don]. *Sci. Hortic.*, **296**: 110920.

Wani, A. A., Zargar, S. A., Malik, A. H., Kashtwari, M., Nazir, M., Khuroo, A. A., ... & Dar, T. A. (2017). Assessment of variability in morphological characters of apricot germplasm of Kashmir, India. *Sci. Hortic.*, **225**: 630-637.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.08

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Development of Cryoconservation Protocol for *In-vitro* Shoot Bases of *Allium ampeloprasum* L. for Long-term Conservation

Ravi Gowthami[1§], Subhash Chander[1§], Ruchira Pandey[1], Muthusamy Shankar[2] and Anuradha Agrawal[1,3*]

Abstract

Critical factors for the development of cryo conservation protocol for *Allium ampeloprasum* L. conserved in the *in-vitro* Genebank (IVGB) at ICAR-NBPGR of India were investigated. Shoot bases (1.0 x 1.5 mm) excised from 8-wk-old shoot cultures grown on growth media (MS + 0.1 mg/l NAA + 0.02 mg/l 2iP + 0.3 M sucrose) and pregrown at 22/ 5°C for 16/8 hours alternate temperature regime, precultured on MS medium supplemented with 0.3 M sucrose at standard culture conditions (SCC) for 16 hours followed by loading solution treatment for 60 minutes, PVS2 dehydration for 40 minutes were successfully cryoconserved using vitrification and droplet-vitrification technique. Droplet-vitrification technique improved post-thaw regrowth by ~58.33% as compared to vitrification. Thus, a standardized protocol was used for cryobanking of shoot bases of *A. ampeloprasum*.

Keywords: *Allium ampeloprasum*, Vitrification, Droplet-vitrification, Shoot bases, Cryobanking.

[1]Tissue Culture & Cryopreservation Unit, ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India

[2]Division of Plant Genetic Resources, ICAR-Indian Agricultural Research Institute, New Delhi, India

[3]National Agricultural Higher Education Project (NAHEP), Krishi Anusandhan Bhawan-II, Indian Council of Agricultural Research (ICAR), Pusa Campus, New Delhi, India

[§]Authors contributed equally to this work and shares first authorship

***Author for correspondence:**

Anuradha.Agrawal@icar.gov.in

Received: 29/03/2023 **Revised:** 25/05/2023

Accepted: 26/05/2023

How to cite this article: Gowthami, R., Chander, S., Pandey, R., Shankar, M., Agrawal, A. (2023). Development of Cryoconservation Protocol for *In-vitro* Shoot Bases of *Allium ampeloprasum* L. for Long-term Conservation. Indian J. Plant Genetic Resources. 36(3), 396-401. **DOI:** 10.61949/0976-1926.2023.v36i03.08

Introduction

Genus *Allium* is one of the largest monocot genera comprising >1,100 taxa present globally, and India has a rich diversity of this genus (Pandey *et al.*, 2022). Since 1986, efforts have been made towards collecting and conservating cultivated and wild *Alliums* (Pandey *et al.*, 2022). In India, *Allium* genetic resources conservation is achieved through a systematic *trans-situ* approach and a total of 5,349 germplasm accessions of *Allium* genetic resources (~20 species) (both indigenous and exotic collections) are conserved in National Genebank at ICAR-NBPGR, New Delhi in its Seed genebank, *in-vitro* gene bank (IVGB), Cryogenebank, and Field genebank at NBPGR regional station, Bhowali (Anuradha *et al.*, 2023). The species *Allium ampeloprasum* L. (syn *A. lineare* Mill.) is a species with an onion-like flavor. The species originated in Middle Asia, with secondary centres of development and distribution in Western Asia and the Mediterranean countries (Swami and Veeregowda, 2006). The etymology of specific epithet is from Greek words '*ampelos*' connoting 'vineyard' and 'prason' meaning 'leek'. This species has multifarious uses, *viz.,* consumed as raw vegetable, as ingredient in many foods, also used in preparation of herbal medicines due to its immense medicinal properties like antioxidant activity, antimicrobial activity, hepatoprotective activity, anticancer activity, anti-osteoporotic activity, antidiabetic activity, anti-inflammatory activity, gastroprotective activity, anti-hypercholesterolemic/hypolipidemic activity, immunomodulatory activity, platelet anti-aggregation activity spasmolytic activity, insecticidal and antifungal properties (Lim, 2015). Although the

species produces seeds, due to its outbreeding nature, cryopreservation has been suggested as a viable method of long-term conservation of its germplasm (Volk *et al*., 2004).

A. ampeloprasum accession EC328492 was procured from the United States Department of Agriculture- Agricultural Research Service, USDA-ARS (Variety: PI-369525) and this species was conserved in field genebank, which is prone to biotic and abiotic stresses; germplasm conserved in the IVGB at ICAR (Indian Council of Agricultural Research) - National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India for over two decades requires periodic subculturing at 16-week intervals, which is labor intensive and time-consuming and only provides short- to medium-term storage (Pandey *et al*., 2022). Hence, there is a need for safe, cost-effective and long-term conservation of this species. So far, cryo conservation is the only available method for long-term conservation of plant genetic resources (PGR) (Agrawal *et al*., 2022a; Sharma *et al*., 2020). In our laboratory we had developed cryo conservation protocols for *in-vitro* conserved germplasm of several species (Agrawal *et al*., 2022b) *viz*., *Bacopa monnieri* (Sharma *et al*., 2011, 2017), Gowthami *et al*., 2023a), Dahlia (Gowthami *et al*., 2023b), *Dioscorea deltoidea* (Dixit-Sharma *et al*., 2005; Mandal and Dixit, 2000; Mandal and Dixit-Sharma, 2007; Sharma *et al*., 2022), *Fragaria* × *ananassa* (Gupta and Tewari, 2020), *Gentiana kurroo* (Sharma *et al*., 2021), *Musa* spp. (Agrawal *et al*., 2004, 2008, 2014), *Picrorhiza kurroa* (Sharma and Sharma, 2003) and *Rubus* spp. (Gupta and Reed, 2006). So far, no reports exist on the cryoconservation of *A. ampelprasum*. With this background, the present study was formulated with the objectives (a) to investigate the critical factors for cryoconservation of *in-vitro* shoot bases; (b) to develop an efficient cryoconservation protocol for cryobanking of *A. ampeloprasum*.

Material and Methods

Plant Material

In-vitro shoot cultures of *A. ampeloprasum* accession EC328492 maintained in the i*n-vitro* Gene Bank (IVGB) of ICAR- NBPGR, New Delhi, India, were utilized as mother culture.

Establishment of *In-vitro* Cultures

Initially, the *in-vitro* grown plants were cultured on shoot multiplication medium (SM) comprising Murashige and Skoog medium (MS) supplemented with 0.1 mg/l naphthalene acetic acid (NAA), 0.02 mg/l 6-γ,γ-dimethylallylamino purine (2iP) (Sigma-Aldrich, St. Louis, MO), 3% (w/v) sucrose (HiMedia Laboratories, Mumbai, India) with pH adjusted to 5.8 prior to autoclaving and solidified using 0.8% (*w/v*) agar. Shoot bases of 1.0 to 1.5 cm were excised in a sterile laminar flow chamber and inoculated in each culture tube and cultures were maintained under standard culture room conditions (SCC) comprising 25 ± 2 °C, 16/8 hours photoperiod, and a light intensity of 40 μEm^{-2} s^{-1} using white fluorescent light (Philips, India). Subculture was performed every 4 to 6 weeks by transferring to fresh medium to generate sufficient explants for cryoconservation experiments.

Shoot base excision

In-vitro shoots were dissected under a stereo zoom microscope (Olympus India, Haryana, India). Initially, the top portion of the shoots were cut, followed by removing 3 or 4 layers of the shoot bases until the transparent shoot base was visible with 1.0 to 1.5 mm size (Figure 4A). Shoot bases were cryoconserved using vitrification (V) and droplet-vitrification (DV) techniques as described below. Initially, different parameters were standardized using the vitrification technique.

Vitrification

The excised shoot bases were precultured on MS medium supplemented with 0.3 M sucrose in 60 mm diameter petri dishes (Hi-Media®) (10 mL medium/Petridish) at SCC for 16 hours. Precultured shoot bases were placed carefully in 1.8 mL Nunc™ cryovials (Thermo Fisher Scientific®, Roskilde, Denmark) containing loading solution (LS) [liquid MS medium + 2.0 M glycerol (Sigma-Aldrich, St. Louis, USA) + 0.4 M sucrose] for 60 minutes at room temperature (25 ± 2°C) under sterile laminar flow chamber. Thereafter, LS was removed using a Pasteur pipette and 1.0 mL of pre-chilled plant vitrification solution 2 (henceforth referred as PVS2 solution) was added. PVS2 comprising 0.4 M sucrose, 30% (*w/v*) glycerol, 15% (*w/v*) ethylene glycol and 15% (*w/v*) dimethyl sulfoxide (Sigma-Aldrich, St. Louis, USA) in MS medium (pH 5.8) (Sakai *et al*., 1990) was incubated for 0, 10, 20, 30, 40, 50 and 60 minutes (depending on experiment) at room temperature (25 ± 2°C). After the desired period of PVS2 dehydration, cryovials with the shoot bases in PVS2 solution were placed in cryocanes, immediately plunged into liquid nitrogen (LN) in a Dewar flask, and retained for at least 1-hour. For thawing, cryovials were removed from LN and rewarmed rapidly by immediately plunging in warm water at 42°C for 2 minutes with constant stirring. After thawing, PVS2 solution was quickly removed using a Pasteur pipette and the shoot bases were rinsed 4 to 5 times with unloading solution (US; 1.2 M sucrose in liquid MS medium; pH 5.8) for 20 minutes at room temperature.

Effect of toxicity of PVS2 dehydration duration

For this experiment, shoot bases were excised from 4-week-old cultures pregrown on SM medium and precultured on MS + 0.3 M sucrose for 16 hours under SCC. Precultured shoot bases were subjected to LS treatment for 60 minutes, followed by different PVS2 dehydration durations (10, 20, 30, 40, 50 and 60 minutes) and US treatment for 20 minutes.

Effect of pregrowth duration, media and temperature

For this experiment, shoot cultures were pregrown for 4, 6 and 8 weeks on SM and MS medium supplemented with 0.1 mg/l NAA, 0.02 mg/l 2iP (Sigma-Aldrich, St. Louis, MO), 0.3 M (*w/v*) sucrose (HiMedia Laboratories, Mumbai, India) with pH adjusted to 5.8 prior to autoclaving and solidified using 0.8% (w/v) agar (hereafter called as SM10 medium) and incubated under 25 ± 2°C and 22/5°C. For pregrowth under 25 ± 2°C, cultures were maintained in a culture room with SCC and for pregrowth at 22/5°C, cultures were maintained in a biological oxygen demand (BOD) incubator (Ocean Life Science Corporation, India) for 16 hours of 22°C and 8 hours of 5°C, 16/8 hours of photoperiod using white fluorescent light.

Droplet-Vitrification

The excised shoot bases were precultured on MS medium supplemented with 0.3 M sucrose in 60 mm diameter Petri dishes at SCC for 16 hours and treated with LS (60 minutes) and PVS2 (40 minutes) as followed in the vitrification protocol. Before 2 minutes of the completion of PVS2 dehydration, 10 shoot bases were placed on a 5 µL droplet of PVS2 solution on a sterile aluminium foil strip (20 × 5 mm). Subsequently, the aluminium strip with shoot bases was plunged directly in LN for a few seconds and later placed into cryovial (1.8 mL) (filled with LN) in a polycarbonate cryo-box, held in a thermocol ice box, for at least an hour. For rewarming, aluminium strips were removed from LN, immediately placed into the US solution in a petri plate (35 mm), and incubated for 20 minutes at room temperature. In both techniques, shoot bases treated with all the solutions (LS and PVS2) but not frozen in LN and rinsed with US for 20 minutes, are treated as controls.

Regrowth and In-vitro Plantlet Development

After US treatment, shoot bases were placed on sterile dry filter papers placed in petri plates to remove the adhered solution. Later shoot bases were placed to 60 mm petridishes containing regrowth medium (SM medium) (10 mL medium/ petridish) and incubated in dark (covered with aluminum foil) at 25 ± 2°C for five days to avoid photo-oxidation. Thereafter, the petri plates with shoot bases were shifted to SCC. After 6 weeks, surviving shoot bases were transferred in culture tubes with SM medium (150 x 25 mm) and incubated under SCC. Regrowth was determined by dividing the shoot bases regrowing into normal shoots after four weeks of plating by the total number of shoot bases inoculated and expressed as a percentage.

Statistical Analysis

All the experiments were carried out in using completely randomized design (CRD) repeated thrice with 20 shoot bases in each replication. Analysis of variance (ANOVA) and duncan's multiple range test (DMRT) was used for comparison and for significant differences among means ($p \leq 0.05$) using SPSS statistics version 22.0 software package. Prior to analysis, the original percentage data were arcsin transformed ($y' = \arcsin y^{1/2}$) to stabilize the variance of data. Results are presented as mean (%) ± standard error (SE). Graphs were prepared by using Microsoft Excel 2010.

Results and Discussion

Cryoconservation is the only available method for safe and long-term conservation of plant genetic diversity. It is being used in many genebanks across the globe, as a complementary conservation strategy for backup to field and/or *in-vitro* collections to safeguard against loss of PGR (Agrawal *et al.*, 2022a; Sharma *et al.*, 2019, 2020). So far, in *Alliums*, cryopreservation (garlic and shallots) was attained using shoot bases, bulbils excised from field cloves which eliminates the establishment of *in-vitro* cultures following vitrification, droplet-vitrification and encapsulation-dehydration (Wang *et al.*, 2020 and references therein). However, contamination is the major encounter through this procedure, many other species do not produce bulbils and also obtaining sufficient explants is very difficult. Hence, in the present study we attempted cryoconservation using *in-vitro* derived shoot bases. The development of cryo conservation protocol involves a series of events and protocols are species/genotype-specific. Hence, in each species, standardization of different parameters *viz.,* explant size, age of mother plants, cold hardening, preculture media and duration, cryopreservation technique, regrowth media etc. are required (Zamecnik *et al.*, 2021). Accordingly, different critical parameters were tested in the present study to develop an efficient cryoconservation protocol for *A. ampeloprasum*.

In the first set of experiments, shoot bases were exposed to six different PVS2 dehydration durations to optimize cryoprotectant treatment duration. No shoot tips

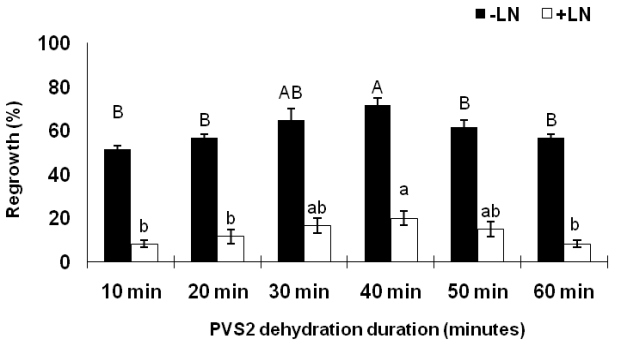


Figure 1: Effect of PVS2 dehydration duration on regrowth of non-cryoconserved (-LN) and cryoconserved (+LN) shoot bases. Data are presented in vertical bars represent mean ± SE. Significant differences ($P \leq 0.05$) in regrowth among non-cryoconserved shoot bases (upper case) and among cryoconserved shoot bases (lower case) are presented by different alphabets analyzed by Duncan's Multiple Range Test

survived cryoconservation without PVS2 treatment, and PVS2 dehydration duration significantly influenced the regrowth of both cryoconserved and non-cryoconserved shoot bases (Figure 1). Gradual increase in regrowth was observed when shoot bases treated with PVS2 solution for 10 to 40 minutes *i.e.,* 51.7 to 71.7% (-LN) and 8.33 to 20% (+LN). However, further increase in PVS2 duration to 50 and 60 minutes resulted in significant decrease in regrowth to 61.7 and 56.7% of non-cryoconserved and 15 and 8.33% of cryoconserved shoot bases. A similar toxic effect of lower and higher PVS2 treatment duration on post-thaw regrowth was reported by Sharma *et al.* (2021) in *Gentiana kurroo*. Hence, for further experiments to improve regrowth, PVS2 dehydration for 40 minutes was followed. Optimization of treatment duration of explants with cryoprotectants is one of the major critical steps to enhance the tolerance to LN exposure and among several cryoprotectants, PVS2 used in many species (Zamecnik *et al.*, 2021). A decrease in the optimum treatment time has been shown to damage explants after LN exposure due to intra- and inter-cellular ice crystal formation and extended period treatment damages explants due to chemical toxicity (Zamecnik *et al.*, 2021).

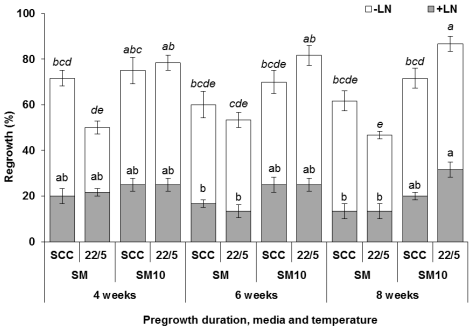


Figure 2: Effect of pregrowth duration, media and temperature on regrowth (%) of non-cryoconserved (-LN) and cryoconserved (+LN) shoot bases. Data are presented in vertical bars represent mean ± SE. Significant differences ($P \leq 0.05$) in regrowth among non-cryoconserved shoot bases (lower case italicized) and among cryoconserved shoot bases (lower case non-italicized) are presented by different alphabets analyzed by Duncan's Multiple Range Test

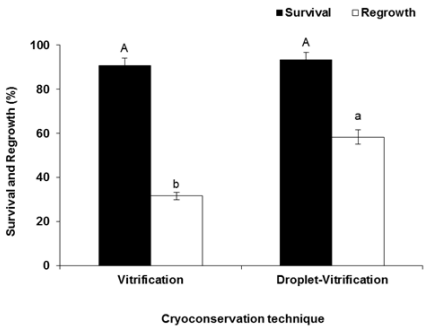


Figure 3. Comparison of two cryoconservation techniques (vitrifcation and droplet-vitrification) on post-thaw regrowth of shoot bases. V- Vitrification, DV- Droplet-Vitrification. Data are presented in vertical bars represent mean ± SE. Significant differences ($P \leq 0.05$) in survival (upper case) and regrowth (lower case) are presented by different alphabets analyzed by Duncan's Multiple Range Test

Induction of dehydration tolerance of explants before before exposure to LN is an essential step in cryoconservation, which is generally achieved by pregrowth of mother plants and preculture of explants, either at low temperature or different temperature regimes, using high sucrose media (commonly 0.3 M sucrose) and growth regulators (Suzuki *et al.*, 2005; Reinhoud *et al.*, 2000; Bettoni *et al.*, 2021). Hence in the present work, to increase the post-thaw regrowth, experiments were conducted to identify the optimum pregrowth duration, pregrowth media and pregrowth temperature to enhance the tolerance to LN freezing and results are presented in Figure 2. Explants survived (46.67–86.67%) and exhibited normal growth in non-frozen controls. These three factors have a significant effect on post-thaw regrowth. It was observed that when shoot bases were excised from 4-week-old cultures pregrown on multiplication medium had 20% regrowth, which increased to 25% when pregrown on shoot multiplication medium with 0.3 M sucrose (SM10) maintained at SCC. Shoot bases excised from 6-week-old cultures pregrown on multiplication medium, resulted in reduction of post-thaw regrowth (16.67%), which further decreased to 13.33% when maintained at 25 ± 2°C. However, similar regrowth of 25% was obtained, when pregrown on SM10 medium maintained at SCC. Similarly, a further increase in pregrowth duration to eight weeks had positive direction improvement when pregrown on SM10 medium at 25/5°C resulting in increase in post-thaw regrowth to 31.67% in comparison to 20% (pregrown on SM10 maintained at SCC). In contrast, negative direction regrowth was observed when pregrown on SM medium and cultures maintained at SCC. From this study it is evident that, pregrowth duration from 8-week-old cultures in comparison to 4 weeks and 6 weeks, pregowth of cultures on shoot multiplication medium with 0.3 M sucrose instead of shoot multiplication medium with 3% sucrose and pregrowth temperature of 25/5°C instead of SCC significantly increased post-thaw regrowth (10%). Thus, the experiment clearly indicated that excision of shoot bases from 8-week-old cultures pregrown on multiplication media supplemented with 0.3 M sucrose and pregrown at two alternate temperature regimes *i.e,* 25/5°C for 16/8 hours has increased the osmotolerance further to enhance the tolerance to LN exposure. Positive impact of pregrowth at low temperatures on post-thaw recovery has been reported in apple, pear, *Rubus, Prunus*, kiwifruit, birch and mulberry (Suzuki *et al.*, 2005). In conformity with previous reports, pregrowth at low temperature of 5°C for 8 hours, alternatively with 22°C for 16 hours enhanced the post-thaw regrowth. Yoon *et al.* (2006) found that cold hardening, the addition of osmotic agents, culture conditions such as high light intensity, ventilation of culture vessels, and low planting density led to increased post-thaw survival in potato.

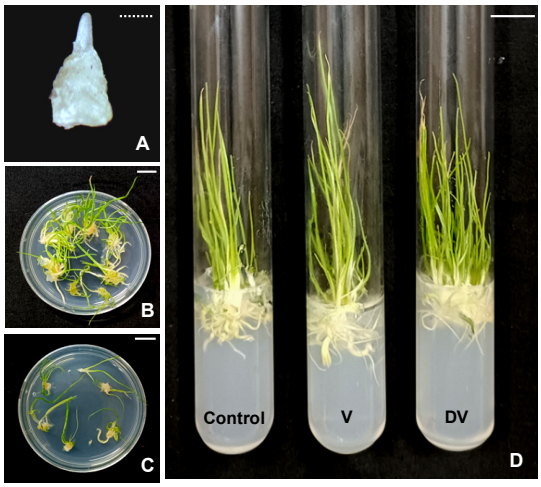


Figure 4: Cryoconservation of *in-vitro* shoot bases of *Allium ampeloprasum* L. using vitrification and droplet-vitrification techniques. (A) Shoot bases for cryoconservation (1.5 mm x 1.0 mm), (B) Control (+PVS2, -LN of DV protocol), (C) Post-thaw regrowth of shoot bases after droplet-vitrification, (D) *In-vitro* plantlet development Control: PVS2, -LN, V: post-thaw regenerated shoot bases of vitrification protocol, DV: post-thaw regenerated shoot bases of droplet-vitrification protocol. Scale bar: dotted line (0.5 mm), solid line (1.0 cm)

Figure 3 presents the feasibility of DV technique using parameters standardized for vitrification technique. In the present study, irrespective of technique, the non-frozen controls (but PVS2 treated) exhibited 100% survival (data not presented) (Figure 4). As reflected in Figure 3. comparison of vitrification and droplet-vitrification on post-thaw regrowth showed significant differences based on DMRT ($P \leq 0.05$). DV technique resulted in significant improvement in post-thaw regrowth (58.33%) (Figure 4C), which was almost twice as compared to vitrification (31.67%). The increased post-thaw survival or regrowth using DV technique in comparison to vitrification has been reported in many species (Agrawal *et al.*, 2004; Panis *et al.*, 2005; Park and Kin, 2015; Benelli *et al.*, 2021; Sharma *et al.*, 2021). The cryoconserved shoot bases produced shoots without transitory callus formation, similar to non-treated controls. All the survived shoot bases developed into plantlets *in-vitro* (Figure 4D).

Conclusion

To the best of our knowledge, this is the first report on cryoconservation protocol development for *Allium ampeloprasum* shoot bases. Droplet-vitrification technique improved post-thaw regrowth by ~58.33% as compared to vitrification. Thus, a standardized protocol was successfully used for cryobanking of *A. ampeloprasum* accession EC328492.

Acknowledgement

We thank Director, ICAR-National Bureau of Plant Genetic Resources, New Delhi, for facilities and encouragement. Technical assistance provided by Mr Rakesh and Mr Anil is greatly acknowledged.

References

Agrawal, A., Gowthami, R., Srivastava, V., & Chander, S. (2023). *Trans situ* conservation of PGR. In: RK Gautam et al., (Eds.) *Plant Genetic Resources Management: Theory and Practices. The Director, ICAR-NBPGR, New Delhi, India*, pp 44-52.

Agrawal, A., Swennen, R., & Panis, B. (2004). A comparision of four methods for cryopreservation of meristems in banana (*Musa* spp.). *CryoLetters*, **25(2)**: 101-110.

Agrawal, A., Tyagi, R. K., & Goswami, R. (2008). Cryopreservation of subgroup Monthan (ABB) of Indian cooking banana (*Musa* spp.) germplasm. *Curr. Sci.*, **94(9)**: 1125-1128.

Agrawal, A., Sanayaima, R., Singh, R., Tandon, R., Verma, S., & Tyagi, R. K. (2014). Phenotypic and molecular studies for genetic stability assessment of cryopreserved banana meristems derived from field and *in vitro* explant sources. *In In Vitro Cell. Dev. Biol. Plant.*, **50**: 345-356.

Agrawal, A., Gowthami, R., Chander, S., & Srivastava, V. (2022a). Sustainability of *In Vitro* Genebanks and Cryogenebanks. *Indian J. Plant Genet. Resources*, **35(3)**: 180-184.

Agrawal, A., Sharma, N., Gupta, S., Bansal, S., Srivastava, V., Malhotra, E. V., & Singh, K. (2022b). Biotechnological applications for plant germplasm conservation at ICAR-National Bureau of Plant Genetic Resources, India- recent achievements. *Acta Hortic.*, **1339**: 29-42.

Benelli, C., Carvalho, L. S., El Merzougui, S., & Petruccelli, R. (2021). Two advanced cryogenic procedures for improving *Stevia rebaudiana* (Bertoni) cryopreservation. *Plants*, **10(2)**: 277.

Bettoni, J. C., Bonnart, R., & Volk, G. M. (2021). Challenges in implementing plant shoot tip cryopreservation technologies. *Plant Cell, Tissue and Organ Culture (PCTOC)*, **144(1)**: 21-34.

Dixit-Sharma, S., Ahuja-Ghosh, S., Mandal, B. B., & Srivastava, P. S. (2005). Metabolic stability of plants regenerated from cryopreserved shoot tips of *Dioscorea deltoidea*–an endangered medicinal plant. *Sci. Hortic.*, **105(4)**: 513-517.

Gowthami, R., Sharma, N., Chandra, R., Kurian, J. S., Malhotra, E. V. & Agrawal, A. (2023a). Development of an improved and simple shoot tip cryoconservation protocol for cryobanking of *Bacopa monnieri* (L.) Wettst. germplasm. *In Vitro Cell. Dev. Biol. Plant*, 1-13.

Gowthami, R., Chander, S., Pandey, R., Shankar, M., & Agrawal, A. (2023b). Development of efficient and sustainable droplet-vitrification cryoconservation protocol for shoot tips for long-term conservation of *Dahlia* germplasm. *Sci. Hortic.*, **321**: 112329.

Gupta, S., & Reed, B. M. (2006). Cryopreservation of shoot tips of blackberry and raspberry by encapsulation-dehydration and vitrification. *CryoLetters*, **27(1)**: 29-42.

Gupta, S., & Tewari, P. (2020). Cryopreservation of Fragaria × ananassa using different techniques. *Acta Horticulturae*, **1298**: 161-166.

Lim, T. K., & Lim, T. K. (2015). *Allium ampeloprasum*. *Edible Medicinal and Non-Medicinal Plants: Volume 9, Modified Stems, Roots, Bulbs*, 103-123.

Mandal, B. B., & Dixit, S. (2000). Cryopreservation of shoot-tips of *Dioscorea deltoidea* Wall. -an endangered medicinal plant. *IPGRI Newsletter for Asia, the Pacific and Oceania*, w, 23.

Mandal, B. B., & Dixit-Sharma, S. (2007). Cryopreservation of in vitro shoot tips of *Dioscorea deltoidea* Wall., an endangered

medicinal plant: effect of cryogenic procedure and storage duration. *CryoLetters*, **28(6)**: 461-470.

Pandey, A., Malav, P. K., Semwal, D. P., Chander, S., Gowthami, R., & Rai, K. M. (2022). Repository of *Allium* Genetic Resources at ICAR-NBPGR: Prospects and Challenges for Collection and Conservation. *Indian J. Plant Genet. Resources*, **35(03)**: 185-190.

Panis, B., Piette, B. M. A. G., & Swennen, R. (2005). Droplet vitrification of apical meristems: a cryopreservation protocol applicable to all Musaceae. *Plant Sci.*, **168(1)**: 45-55.

Park, S. U., & Kim, H. H. (2015). Cryopreservation of sweet potato shoot tips using a droplet-vitrification procedure. *CryoLetters*, **36(5)**: 344-352.

Reinhoud, P. J., Versteege, I., Kars, I., Iren, F. V., & Kijne, J. W. (2000). Physiological and molecular changes in tobacco suspension cells during development of tolerance to cryopreservation by vitrification. In *Cryopreservation of tropical plant germplasm: current research progress and application. Proceedings of an international workshop, Tsukuba, Japan, October, 1998* (pp. 57-66). International Plant Genetic Resources Institute (IPGRI).

Sakai, A., Kobayashi, S., & Oiyama, I. (1990). Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* Osb. var. *brasiliensis* Tanaka) by vitrification. *Plant Cell Reports*, *9*(1), 30-33.

Sharma, N., & Sharma, B. (2003). Cryopreservation of shoot tips of *Picrorhiza kurroa* Royle ex Benth., an indigenous endangered medicinal plant, through vitrification. *CryoLetters*, **24(3)**: 181-190.

Sharma, N., Satsangi, R., & Pandey, R. (2011). Cryopreservation of shoot tips of *Bacopa monnieri* (L.) Wettst by vitrification technique. *Acta Hortic.*, **908**: 283-288.

Sharma, N., Singh, R., Pandey, R., & Kaushik, N. (2017). Genetic and biochemical stability assessment of plants regenerated from cryopreserved shoot tips of a commercially valuable medicinal herb *Bacopa monnieri* (L.) Wettst. *In Vitro Cell. Dev. Biol. Plant*, **53***:* 346-351.

Sharma, N., Pandey, R., & Gowthwami, R. (2020). *In vitro* conservation and cryopreservation of threatened medicinal plants of India. In: Rajasekharan PE, Wani SH (Eds.) *Conservation and utilization of threatened medicinal plants*. Springer, India, pp 181–228.

Sharma, N., Gowthami, R., & Pandey, R. (2019). Synthetic seeds: a valuable adjunct for conservation of medicinal plants. In: Faisal M, Alatar AA (Eds) *Synthetic seeds: germplasm regeneration, preservation and prospects*. Springer International Publishing, Cham, pp. 181–216.

Sharma, N., Gowthami, R., Devi, S. V., Malhotra, E. V., Pandey, R., & Agrawal, A. (2021). Cryopreservation of shoot tips of *Gentiana kurroo* Royle–a critically endangered medicinal plant of India. *Plant Cell, Tissue and Organ Culture (PCTOC)*, **144**: 67-72.

Sharma, N., Malhotra, E. V., Chandra, R., Gowthami, R., Sultan, S. M., Bansal, S., & Agrawal, A. (2022). Cryopreservation and genetic stability assessment of regenerants of the critically endangered medicinal plant *Dioscorea deltoidea* Wall. ex Griseb. for cryobanking of germplasm. *In Vitro Cell. Dev. Biol. Plant*, **58(4)**: 521-529.

Swamy, K. R. M., & Gowda, R. V. (2006). Leek and shallot. In Peter KV (Ed) *Handbook of Herbs and Spices*. Woodhead Publishing: Cembridge, UK, **3**: 365–389.

Volk, G. M., Maness, N., & Rotindo, K. (2004). Cryopreservation of garlic (*Allium sativum* L.) using plant vitrification solution 2. *CryoLetters*, **25(3)**: 219-226.

Wang, M. R., Zhang, Z., Zámečník, J., Bilavčík, A., Blystad, D. R., Haugslien, S., & Wang, Q. C. (2020). Droplet-vitrification for shoot tip cryopreservation of shallot (*Allium cepa* var. *aggregatum*): Effects of PVS3 and PVS2 on shoot regrowth. *Plant Cell, Tissue and Organ Culture (PCTOC)*, **140**: 185-195.

Yoon, J. W., Kim, H. H., Ko, H. C., Hwang, H. S., Hong, E. S., Cho, E. G., & Engelmann, F. (2006). Cryopreservation of cultivated and wild potato varieties by droplet vitrification: effect of subculture of mother-plants and of preculture of shoot tips. *CryoLetters*, **27(4)**: 211-222.

Zamecnik, J., Faltus, M., & Bilavcik, A. (2021). Vitrification solutions for plant cryopreservation: Modification and properties. *Plants*, **10(12)**: 2623.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.09

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Phenotypic Characterization of Crop Genetic Resources of AA-genome Species in Rice (*Oryza spp.*)

Aswin Mahendran[1,2], Mahesh C. Yadav[1*], Gopala Krishnan S[3], Shailesh Tiwari[1] and Tapan K. Mondal[4]

Abstract

Rice (*Oryza sativa* L.) is vital for food security of global population especially in Asian countries where more than 90% of the worlds rice is produced and consumed. For exploring variability in morpho-qualitative traits in crop wild relatives (CWRs) for genetic improvement of rice cultivars, we characterized 88 accessions of eight *Oryza* species of diploid AA-genome utilizing 20 morpho-qualitative traits. High extent of within and between species phenotypic variation was observed for all the morpho-qualitative traits except anther color. Shannon-Weiner's diversity (H' = 0.96) and Pielou's measure of evenness (J = 0.67) also confirmed the presence of a high level of variability in 19 morpho-qualitative traits for both within and between the *Oryza* species. The greater diversity (H' >1.00 to 1.39) was recorded for 11 qualitative traits, namely, flag-leaf angle, basal-leaf sheath color, culm angle, panicle type, panicle exsertion, awn color, apiculus color, lemma and palea color, seed shattering, threshability and pericarp color. A moderate level of diversity (H' = 0.50 to 0.99) was observed for leaf-blade pubescence, ligule color, ligule shape, auricle color, stigma color, and lemma and palea pubescence. However, growth habit and awning displayed low level of diversity (H' < 0.50). Within species variability assessed in 41 accessions of *O. nivara* and 29 accessions of *O. rufipogon* revealed existence of higher variability, in *O. nivara* accessions (H' = 0.87) as compared to *O. rufipogon* (H' = 0.69) for most of the traits. Ward's distance dendrogram grouped the accessions of CWRs into five major clusters, each containing accessions of three to seven species of *Oryza* AA-genome.

Keywords: AA-genome, *Oryza* species, Phenotypic characterization, Qualitative traits, Wild rice.

[1]Division of Genomic Resources, ICAR-National Bureau of Plant Genetic Resources, New Delhi, India.

[2]The Graduate School, ICAR-Indian Agricultural Research Institute, New Delhi, India.

[3]Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India.

[4]ICAR-National Institute of Plant Biotechnology, New Delhi, India.

***Author for correspondence:**

mahesh.yadav1@icar.gov.in

Received: 10/04/2023 **Revised:** 25/05/2023

Accepted: 25/05/2023

How to cite this article: Mahendran, A., Yadav, M.C., Krishnan, S.G., Tiwari, S., Mondal, T.K. (2023). Phenotypic Characterization of Crop Genetic Resources of AA-genome Species in Rice (*Oryza spp.*) . Indian J. Plant Genetic Resources. 36(3), 402-414. **DOI:** 10.61949/0976-1926.2023.v36i03.09

Introduction

Rice (*Oryza sativa* L.) is one of the oldest domesticated cereals in the world. Rice is known as "wonder grain" because it is staple food for more than half of the world's population. For the ever-increasing human population, genetic improvement plays an important role in matching the enhancement of the production and productivity of rice crop to fulfil growing food demand (Khush, 2013). The current global rice (paddy) production is about 756.3 million tonnes, where India ranks second next to China, producing 178.3 million tonnes (FAO, 2022). To meet the challenge of increased production, the novel allelic variation needs to be explored in wild rice germplasm (Brar and Khush, 2018). The genus *Oryza* includes two cultivated (2 *n* = 24) and 22 wild species (2 *n* = 24, 48) consisting of six diploid (AA, BB, CC, EE, FF and GG) and five tetraploid (BBCC, CCDD, KKLL, HHKK and HHJJ) genome types (Vaughan, 1989; Stein *et al*., 2018). As the genetic variability is limited within the cultivated species of rice, the improvement of rice cultivars depends mainly on exploiting the diversity available in the gene pool of crop wild relatives (CWRs) (McCouch *et al*., 2012, 2013). The *Oryza* species are further divided into three gene pools based on crossability and phylogenetic relationships (Harlan and de Wet, 1971; Vaughan, 1989). The primary genepool consists of two cultivated (*O. sativa* and *O. glaberrima*) and six wild species namely, *O. nivara*, *O. rufipogon*, *O. barthii*, *O.*

longistaminata, *O. glumaepatula* and *O. meridionalis*), all belonging to *Oryza* AA-genome (Vaughan *et al.,* 2005). All eight AA-genome species are diploid, cross compatible and showed homologous pairing in the chromosomes (Vaughan *et al.,* 2008; Alsantely *et al*., 2023).

The wild species of *Oryza* are weedy grass like plants, which are inferior in morphological traits such as poor grain quality, inferior plant type, grain shattering and low grain yield (Xu and Sun, 2021; Fornaseiro *et al*., 2022). Though the wild rice species are inferior in morphological traits, they possess a repertoire of useful genes for resistance to biotic, abiotic stress and yield-related traits (Sanchez *et al*., 2013). The morphological traits of wild species differ markedly from cultivated species such as growth habit, tillering, plant height, leaf size, culm, flowering patterns, panicle exsertion, panicle size, panicle branching, awning, seed traits, and adaptation to different habitats and agronomic traits (Brar and Khush 2018; Stein *et al*., 2018; Gaikwad *et al*., 2020). Understanding the extent and type of genetic variation in wild rice populations may help to identify valuable accessions possessing desirable traits for germplasm conservation and utilization in crop improvement. The qualitative trait-based phenotypic characterization has been utilized in rice (Joseph *et al*., 2007; Kharel *et al*., 2022) and CWRs (Khan *et al*., 2021; Sandamal *et al*., 2021), especially to assess the genetic variation in AA-genome species of genus *Oryza* (Samal *et al*., 2018; Singh *et al*., 2018; Tiwari *et al*., 2020). Shannon-Weiner index has been used frequently to measure the diversity within and between the species utilizing both qualitative and quantitative traits in cultivated and wild rice germplasm (Hein *et al*., 2007; Sow *et al*., 2013; Rabara *et al*., 2014; Lei *et al*., 2018; Rao *et al*., 2021). For the effective utilization of rice germplasm consisting of CWRs in plant breeding programmes, firstly they need to be characterized and evaluated. Therefore, the present study was aimed to characterize wild rice accessions of AA-genome species utilizing morpho-qualitative traits.

Material and Methods

Experimental Materials

The experimental materials consisted of 90 *Oryza* accessions belonging to seven wild, one intermediate and two cultivated species of rice acquired from the Indian National Gene Bank, ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi. The wild rice species mainly consisted of AA genome of genus *Oryza* (88 accessions) and one species of CC genome (*O. Officinalis*; two accessions). Of these AA genome species, 41 accessions were of *O. nivara*, 29 accessions of *O. rufipogon*, five accessions of *O. glaberrima,* three accession of *O. meridionalis,* two accessions each of *O. glumaepatula, O. sativa* f. *spontanea,* and *O. longistaminata,* one accession of *O. barthii* and three accessions are of *O. sativa* (checks). The details of rice germplasm accessions studied are provided in Table 1. Geo-referencing of the wild rice accessions was performed using passport data information using 'DIVA-GIS' software version 7.5 (Hijmans *et al*., 2001). The collection sites of 90 *Oryza* spp. accessions were geo-referenced on world map and also depicted on enlarged India map (Figure 1). The geographic distribution of collection sites and number of collections from different countries, states and districts are provided in Table 2.

Morpho-qualitative Trait-based Characterization

Field experiment was conducted at Experimental Farm, Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi during June to December 2021. The experiment was conducted in Augmented Block Design (ABD), and a 40 × 40 cm distance was maintained between line to line and plant to plant. Five blocks with, each containing 18 test accessions and three checks maintained in ABD. The cultural practices were followed as per the standard recommendations for raising a healthy cultivated rice crop. We utilized morphological descriptors developed by International Rice Research Institute (IRRI's) Standard Evaluation System (2013) and Bioversity International (2007) for the phenotypic characterization of wild rice germplasm. Observations were recorded on 20 qualitative traits *viz.,* growth habit, culm angle, basal-leaf sheath color, flag-leaf angle, leaf-blade pubescence, ligule color, ligule shape, auricle color, panicle type, panicle exsertion, awning, awn color, apiculus color, stigma color, anther color, lemma and palea color, seed shattering, panicle threshability, pericarp color, and lemma and palea pubescence. The data on five randomly chosen plants from each accession were recorded at an appropriate plant growth and development stage.

Statistical Analysis

The dendrogram was constructed based on Ward's distance method using R-software version 3.6.3 (Le *et al*., 2008). The bar diagrams depicting the frequency distributions of morpho-qualitative traits were prepared using MS Excel. The diversity estimates based on morpho-qualitative traits were derived utilizing Shannon-Weiner index (Shannon and Weaver, 1949). The formula used for calculating Shannon-Weiner index is as follows.

$$\text{Shannon-Weiner index (H')} = -\sum_{i=1}^{n} \text{Pi ln Pi}$$

Where n is the number of phenotypic classes for a character, P_i is the relative frequency in the i^{th} class of the j^{th} trait and ln is the natural logarithm of P_i. The extent of diversity was interpreted as < 0.50 value of H': low diversity, 0.50 to 1.00 value of H' : moderate and >1.00 value of H': high diversity in the germplasm. The traits, which showed higher value of Shannon-Weiner index, revealed the existence of greater diversity among the accessions. The distribution of diversity was calculated based on Pielou's measure of evenness

Table 1: Passport data of accessions of *Oryza* species germplasm used in morphological analysis

Sl. No.	*Acc. code*	*Accession number*	*Species name*	*Local name*	*District of collection*	*Latitude*	*Longitude*	*State/Country*
1	WR727	IC283158	*O. nivara*	Jhara	Malkangiri	18.257	81.953	Odisha
2	WR728	IC283160	*O. nivara*	Jhara	Malkangiri	18.257	81.953	Odisha
3	WR729	IC283166	*O. nivara*	Jhara	Malkangiri	18.257	81.953	Odisha
4	WR730	IC283172	*O. nivara*	Jhara	Malkangiri	18.257	81.953	Odisha
5	WR731	IC283176	*O. nivara*	Jhara	Malkangiri	18.257	81.953	Odisha
6	WR732	IC283180	*O. nivara*	Jhara	Malkangiri	18.257	81.953	Odisha
7	WR733	IC283149	*O. nivara*	Jhara	Koraput	18.856	82.734	Odisha
8	WR734	IC283152	*O. nivara*	Jhara	Koraput	18.856	82.734	Odisha
9	WR735	IC283155	*O. nivara*	Jhara	Koraput	18.856	82.734	Odisha
10	WR736	IC283181	*O. nivara*	Jhara	Koraput	18.856	82.734	Odisha
11	WR737	IC282978	*O. nivara*	Balunga	Jharsuguda	21.805	83.952	Odisha
12	WR738	IC282985	*O. nivara*	Jhara	Jharsuguda	21.805	83.952	Odisha
13	WR739	IC309827	*O. nivara*	Jhara	Jharsuguda	21.805	83.952	Odisha
14	WR740	IC256850	*O. nivara*	Jhara	Sambalpur	21.445	84.270	Odisha
15	WR741	IC282989	*O. nivara*	Balunga	Sambalpur	21.445	84.270	Odisha
16	WR743	IC336689	*O. nivara*	Paserbalunga	Balangir	20.866	83.216	Odisha
17	WR744	IC336693	*O. nivara*	Paserbalunga	Balangir	20.298	82.829	Odisha
18	WR745	IC283198	*O. nivara*	Jhara	Kalahandi	19.866	83.109	Odisha
19	WR746	IC336695	*O. nivara*	Paserbalnga	Kalahandi	19.866	83.109	Odisha
20	WR747	IC336699	*O. nivara*	Paser	Kalahandi	19.866	83.109	Odisha
21	WR748	IC283189	*O. nivara*	Jhara	Nabarangapur	19.575	82.336	Odisha
22	WR750	IC283195	*O. nivara*	Jhara	Nabarangapur	19.575	82.336	Odisha
23	WR751	IC282993	*O. nivara*	Balunga	Dhenkanal	20.826	85.526	Odisha
24	WR752	IC257161	*O. nivara*	Jhara	Ganjam	19.374	85.083	Odisha
25	WR753	IC330654	*O. nivara*	Jalidhan	Dakshin Dinajpur	25.365	88.580	West Bengal
26	WR754	IC330657	*O. nivara*	Kawa thekra	Dakshin Dinajpur	25.365	88.580	West Bengal
27	WR755	IC330612	*O. nivara*	Jhara	Malda	25.131	88.087	West Bengal
28	WR756	IC330653	*O. nivara*	Jalidhan	Malda	25.131	88.087	West Bengal
29	WR757	IC330610	*O. nivara*	Jhara	Murshidabad	24.161	88.232	West Bengal
30	WR758	IC330611	*O. nivara*	Palua	Murshidabad	24.161	88.232	West Bengal
31	WR759	IC330595	*O. nivara*	Jhara	Birbhum	23.953	87.665	West Bengal
32	WR760	IC330616	*O. nivara*	Jhara	Birbhum	23.953	87.665	West Bengal
33	WR761	IC330621	*O. nivara*	Pasarhi	Faizabad	23.953	87.665	U.P.
34	WR762	IC272981	*O. nivara*	Pasarhi	Faizabad	26.656	82.001	U.P.
35	WR763	IC272984	*O. nivara*	Pasarhi	Faizabad	26.656	82.001	U.P.
36	WR766	IC332599	*O. nivara*	Dhan	Lucknow	26.687	80.984	U.P.
37	WR767	IC598071	*O. nivara*	Tini	Varanasi	25.316	82.973	U.P.
38	WR768	IC598072	*O. nivara*	Tini	Sonbhadra	24.457	82.993	U.P.
39	WR769	IC598110	*O. nivara*	Beda Dhan	Garhwa	24.154	83.799	Jharkhand
40	WR770	IC598108	*O. nivara*	Beda Dhan	Garhwa	24.040	83.540	Jharkhand
41	WR771	IC598106	*O. nivara*	Beda Dhan	Palamau	24.128	84.185	Jharkhand
42	WR773	IC598078	*O. rufipogon*	Baunadhan	Dakshin Dinajpur	25.371	88.556	West Bengal
43	WR774	IC330666	*O. rufipogon*	Jhadadhan	Uttar Dinajpur	25.480	88.240	West Bengal
44	WR775	IC596548	*O. rufipogon*	-	Nagaon	26.460	92.580	Assam
45	WR776	IC381929	*O. rufipogon*	Uridol	Lakhimpur	27.233	94.116	Assam
46	WR777	IC596545	*O. rufipogon*	-	Lakhimpur	27.370	94.250	Assam
47	WR778	IC596547	*O. rufipogon*	-	Lakhimpur	27.608	94.769	Assam

48	WR779	IC336675	*O. rufipogon*	-	Dhemaji	20.212	84.999	Assam
49	WR780	IC256748	*O. rufipogon*	Balunga	Nayagarh	21.895	83.395	Odisha
50	WR781	IC309826	*O. rufipogon*	Jhara	Jharsuguda	21.805	83.952	Odisha
51	WR782	IC309829	*O. rufipogon*	Jhara	Jharsuguda	21.805	83.952	Odisha
52	WR783	IC256759	*O. rufipogon*	Jhara	Sambalpur	21.445	84.270	Odisha
53	WR784	IC598102	*O. rufipogon*	Boridhan	Lohardaga	23.433	84.647	Jharkhand
54	WR785	IC598103	*O. rufipogon*	Beda dhan	Lohardaga	23.433	84.647	Jharkhand
55	WR787	IC330594	*O. rufipogon*	Jhara	Birbhum	23.953	87.665	West Bengal
56	WR789	IC330627	*O. rufipogon*	Udi	Nadia	23.478	88.520	West Bengal
57	WR790	IC330607	*O. rufipogon*	Jhara	Murshidabad	24.161	88.232	West Bengal
58	WR791	IC330609	*O. rufipogon*	Jhara	Murshidabad	24.161	88.232	West Bengal
59	WR792	IC330668	*O. rufipogon*	Dal	Malda	25.131	88.087	West Bengal
60	WR793	IC283165	*O. rufipogon*	Jhara	Malkangiri	18.257	81.953	Odisha
61	WR794	IC283191	*O. rufipogon*	Jhara	Nabarangapur	19.575	82.336	Odisha
62	WR796	IC336687	*O. rufipogon*	Posari	Balangir	20.298	82.829	Odisha
63	WR798	IC283199	*O. rufipogon*	Jhara	Kalahandi	19.866	83.109	Odisha
64	WR799	IC336703	*O. rufipogon*	Paser	Kalahandi	19.866	83.109	Odisha
65	WR800	IC330672	*O. rufipogon*	Balunga	Puri	19.663	85.510	Odisha
66	WR801	IC330676	*O. rufipogon*	Balunga	Puri	19.663	85.510	Odisha
67	WR802	IC336710	*O. rufipogon*	Pansari	Gajapati	19.184	84.160	Odisha
68	WR803	IC257084	*O. rufipogon*	Danda	Cuttack	20.452	85.700	Odisha
69	WR804	IC362061	*O. rufipogon*	Valunki	Jagatsinghpur	20.223	86.181	Odisha
70	WR805	IC362066	*O. rufipogon*	Utennenditeh	Jagatsinghpur	20.223	86.181	Odisha
71	WR806	IC556030	*O. glaberrima*	-	Bamako	12.639	-8.003	Mali
72	WR807	EC132649	*O. glaberrima*	-	Timbuktu	16.766	-3.002	Mali
73	WR808	EC133117	*O. glaberrima*	-	Kwara	8.966	4.387	Nigeria
74	WR809	EC132675	*O. glaberrima*	-	Sikasso	11.322	-5.698	Mali
75	WR810	EC133087	*O. glaberrima*	-	Kaduna	10.501	7.440	Nigeria
76	WR811	IC556056	*O. meridionalis*	-	katherine	-14.452	132.269	Australia
77	WR812	EC384083	*O. meridionalis*	-	katherine	-14.087	132.487	Australia
78	WR813	EC384124	*O. meridionalis*	-	Tenant creek	-19.645	134.191	Australia
79	WR814	IC301735	*O. glumaepatula*	-	Costa Rica	9.748	-83.753	C. America
80	WR815	IC301738	*O. glumaepatula*	-	Panama	8.538	-80.782	C. America
81	WR816	IC380147	*O. barthii*	-	Ondo	6.914	5.147	Nigeria
82	WR818	IC301734	*O. officinalis*	-	Raipur	21.251	81.629	Chhattisgarh
83	WR819	EC384063	*O. officinalis*	-	Pattaya	12.923	100.882	Thailand
84	WR820	IC627921	*O. sativa* f. *spontanea*	Uri Dholl Ghas	Morigaon	26.110	92.220	Assam
85	WR821	IC627922	*O. sativa* f. *spontanea*	Uri Dholl Ghas	Morigaon	26.060	92.060	Assam
86	WR822	EC133275	*O. longistaminata*	-	Borno	11.884	13.152	Nigeria
87	WR823	EC784830	*O. longistaminata*	-	Jigawa	12.228	9.561	Nigeria
88	Nagina22	IC123319	*O. sativa*	-	Nagina	29.443	78.432	U.P.
89	Pusa44	IC590849	*O. sativa*	-	IARI Delhi	28.633	77.152	Delhi
90	Pusa 1850	IC627102	*O. sativa*	-	IARI Delhi	28.633	77.152	Delhi

Abbreviations: Acc. = Accession; O. = *Oryza*; C. America = Central America; U.P. = Uttar Pradesh

Table 2: Geographic distribution of 90 accessions of *Oryza* species germplasm

Sl. No.	*Species*	*State/Country*	*Site/District of collection*	*Total*
1.	*O. nivara*	Odisha	Malkangiri (6), Koraput (4), Jharsuguda (3), Sambalpur (2), Balangir (2), Kalahandi (3), Nabarangapur (2), Dhenkanal (1) and Ganjam (1)	24
		West Bengal	Dakshin Dinajpur (2), Malda (2), Murshidabad (2) and Birbhum (2)	8
		Uttar Pradesh	Faizabad (3), Lucknow (1), Varanasi (1) and Sonbhadra (1)	6
		Jharkhand	Garhwa (2) and Palamau (1)	3
	Total accessions	4 states	19 districts	41
2.	*O. rufipogon*	West Bengal,	Dakshin Dinajpur (1), Uttar Dinajpur (1), Birbhum (1), Nadia (1), Murshidabad (2), Malda (1)	7
		Assam	Nagaon (1), Lakhimpur (3), Dhemaji (1)	5
		Odisha	Malkangiri (1), Nabarangapur (1), Balangir (1), Kalahandi (2), Puri (2), Gajapati (1), Cuttack (1), Jagatsinghpur (2), Nayagarh (1), Jharsuguda (2), Sambalpur (1)	15
		Jharkhand	Lohardaga (2)	2
	Total accessions	4 states	17 districts	29
3.	*O. glaberrima*	Mali	Bamako (1), Timbuktu (1), Sikasso (1)	3
		Nigeria	Kwara (1), Kaduna (1)	2
4.	*O. meridionalis*	Australia	Katherine (2), Tenant creek (1)	3
5.	*O. glumaepatula*	C. America	Costa Rica (1), Panama (1)	2
6.	*O. barthii*	Nigeria	Ondo (1)	1
7.	*O. officinalis*	Chhattisgarh	Raipur	1
		Thailand	Pattaya	1
8.	*O. sativa* f. *spontanea*	Assam	Morigaon (2)	2
9.	*O. longistaminata*	Nigeria	Borno (1), Jigawa (1)	2
10.	*O. sativa* (Checks)	Delhi	New Delhi (3)	3
	Total accessions	7 countries	-	20

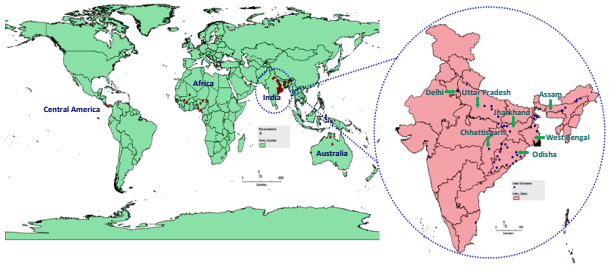


Figure 1: World map showing geo-referencing of 96 accessions of nine *Oryza* species. Enlarged India map depicting distribution of various accessions of wild rice species in seven states of Indian Union.

(Pielou, 1966). The value of evenness gives equality in the distribution of traits among the accessions.

Results

Morphological Variation for Qualitative Traits

The data of 20 morpho-qualitative traits was recorded in 90 accessions of 10 *Oryza* species using IRRI and Bioversity International descriptors. Bar diagrams depicting frequency distribution and morphological variability for 15 qualitative traits are presented vide Figure 2. A wide range of variability was observed for all the morpho-qualitative traits between different accessions within species and between accessions of different species (Figure 3). The growth habit of wild rice accessions varied from annual to perennial types. Out of 90 accessions, 94.5% of the accessions exhibited annual growth habit and 5.5% of the accessions showed perennial growth habit. Among the morpho-qualitative traits, the highest variation was observed for culm angle. Five alternative forms of culm angles were erect, semi-erect, open, spreading and procumbent. Most of the accessions displayed semi-erect (40.0%) type of culm angle followed by erect type (31.1%). The procumbent type of culm angle was observed only in five accessions of *O. rufipogon*. The green color of basal-leaf sheath was the prominent one (48.9%) followed by light purple (30.0%) and purple (12.2%) colors (Figures 2b, 3a, b). The purple lines on basal-leaf sheath were observed mostly in *O. nivara* accessions (Figure 3c). Among the wild rice accessions studied, we observed four types of leaf angles in the germplasm. The most prominent type was horizontal (43.3%) followed by erect (28.9%) and

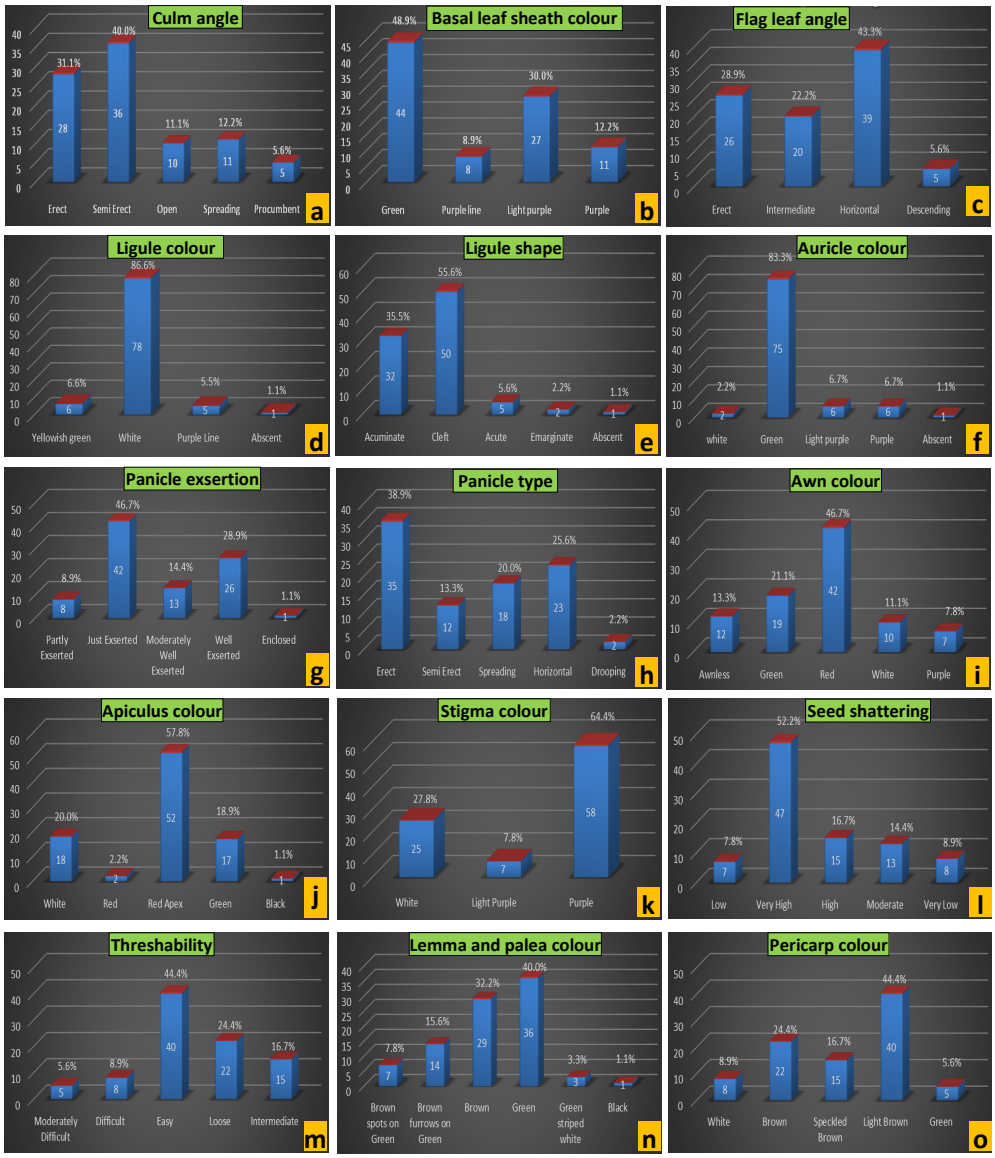


Figure 2: Bar diagrams displaying frequency distribution and phenotypic classes among 90 accessions of *Oryza species* for 15 qualitative traits. (a) Culm angle, (b) Basal-leaf sheath, (c) Flag-leaf attitude, (d) Ligule color, (e) Ligule shape, (f) Auricle color (g) Panicle exsertion, (h) Panicle type, (i) Awn color, (j) Apiculus color, (k) Stigma color, (l) Seed shattering, (m) Threshability, (n) Hull color and (o) Pericarp color.

intermediate (22.2%). The descending type (5.6%) of leaf orientation was the least observed. In cultivated rice, three types of leaf-blade pubescence are found namely, glabrous, intermediate and pubescent. However, the accessions in present study showed only two types with most of the accessions displayed pubescent (80.0%) type of leaf-blade pubescence followed by intermediate (20.0%).

Most of the wild rice accession exhibited white (86.7%) colored ligules followed by yellowish green (6.7%) and purple lines on ligules (5.5%) (Figures 2d, 3d-f). In AA-genome *Oryza* species studied, most of the accessions possessed green colored auricles (83.3%), whereas light purple and purple (6.7% each) (Figures 2f, 3g-i) and white (2.2%) colored auricles found in low frequency. Out of 90 accessions, we found one accession namely WR819 (*O. officinalis*) of liguleless type, while most of the accessions possessed 2-cleft (55.6%) ligule shape followed by acuminate (35.5%), acute (5.6%) and emerginate (2.2%) ligules shapes (Figures 2e, 3j-l). Most of the accessions showed just exserted (46.7%) type of panicle exsertion followed by well-exerted (28.9%) and moderately well-exserted (14.4%) panicles (Figure 2g). However, 8.9% accessions displayed partly exserted and 1.1% of accessions showed enclosed type of panicle exsertion. Erect type was the prominent panicle type and observed in 38.9% of the accessions followed by horizontal (25.6%) and spreading (20.0%) types of panicles (Figure 2h). The semi-erect (13.3%) and drooping type (2.2%, *O. officinalis*) of panicles were observed.

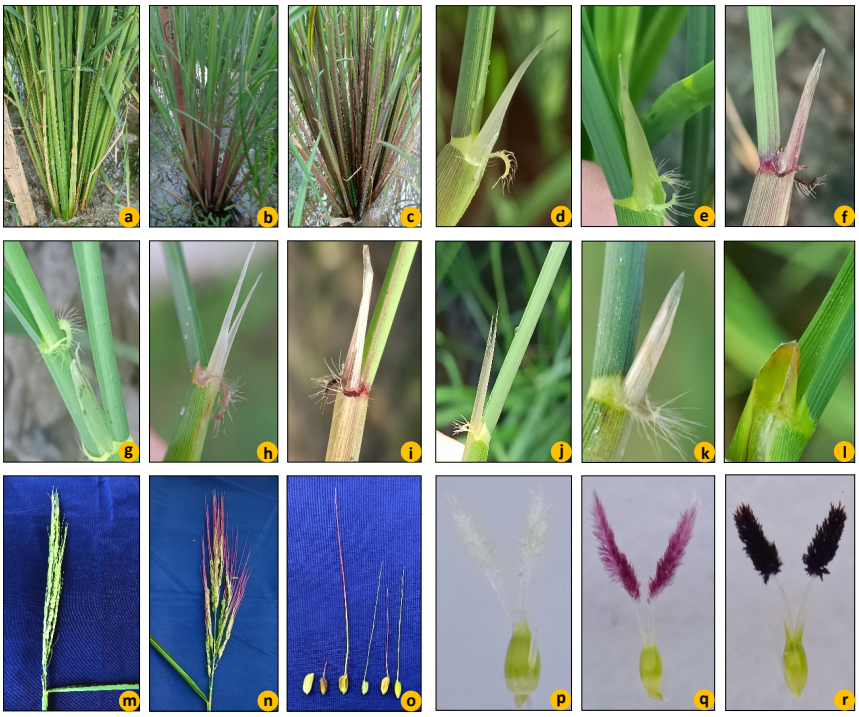


Figure 3: Basal-Leaf sheath color: (a) Green-WR732 (*O. nivara*), (b) Light Purple-WR809 (*O. glaberrima*), (c) Purple line-WR735 (*O. nivara*); Ligule color: (d) White-WR791 (*O. rufipogon*), (e) Yellowish green-WR733 (*O. nivara*), (f) Purple lines- WR-809 (*O. glaberrima*); Auricle color: (g) Green-WR 740 (*O. nivara*), (h) Light purple-WR809 (*O. glaberrima*; (i) Purple-WR781 (*O. rufipogon*); Ligule shape: (j) 2-Cleft-WR768 (*O. nivara*), (k) Acuminate-WR753 (*O. nivara*), (l) Acute-WR813 (*O. meridionalis*); Awn color: (m) Green-WR823 (*O. longistaminata*), (n) Red-WR782 (*O. rufipogon*), (o) Variations in awn size; Stigma color: (p) White-WR804 (*O. rufipogon*), (q) Light purple -WR743 (*O. nivara*), (r) Purple-WR809 (*O. glaberrima*)

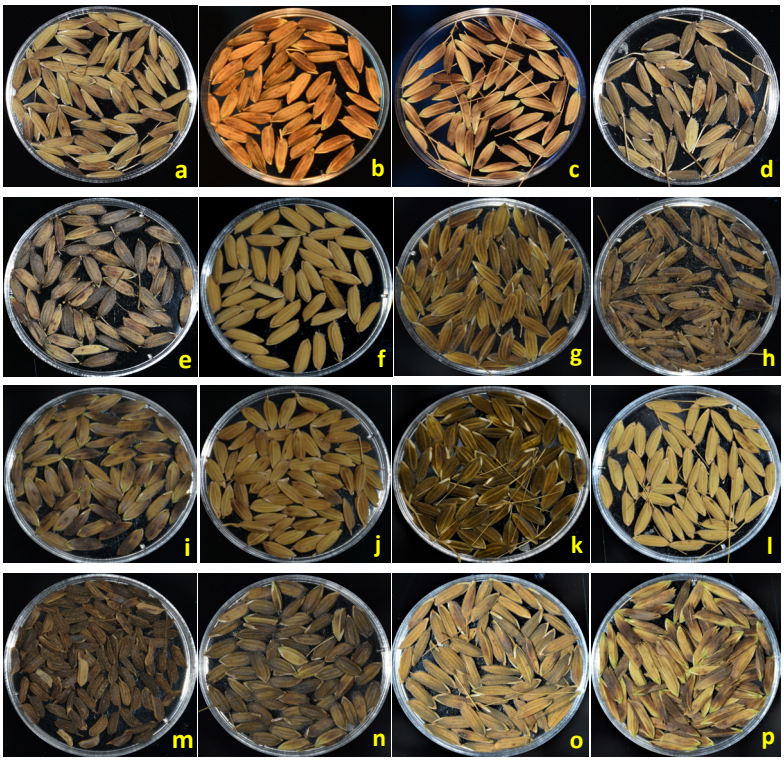


Figure 4: Phenotypic variability for hull color in *Oryza* species after harvest. (a-f) *nivara* (WR727, 728,729, 730, 757, 766), (g-i) *rufipogon* (WR773, 785, 804), (j-K) *glaberrima* (WR806, 807), (l) *meridionalis* (WR811), (m) *officinalis* (WR818), (n-o) *spontanea* (WR820, 821), and (p) *longistaminata* (WR823)

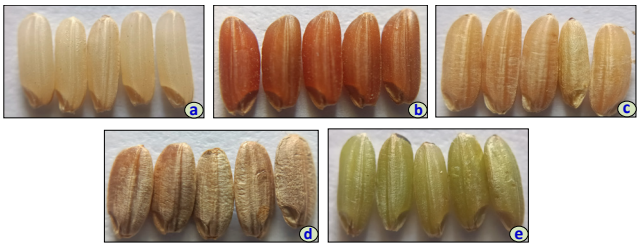


Figure 5: Phenotypic variability for pericarp color in *Oryza* species: (a) White in WR816 (*O. barthii*), (b) Brown in WR811 (*O. meridionalis*), (c) Light Brown in WR808 (*O. glaberrima*), (d) Speckled brown in WR732 (*O. nivara*) and w Green in WR799 (*O. rufipogon*)

Awning is a primitive character mostly found in CWRs of rice. The wild rice accessions exhibited awning phenotype (86.7%), whereas awnless phenotype (13.3%) was observed mainly in cultivated rice (Figure 3o). Amongst the four awn color variation observed, red colored awns (46.7%) were the most frequent followed by green (21.1%), white (11.1%) and purple (7.8%) (Figures 2i, 3m, n). Majority of the accessions displayed red apiculus color (57.8%) followed by white (20.0%) and green apiculus (18.9%) colors. However, red apex found in 2.2% accessions (WR731 and WR768; *O. nivara*) and black apiculus color in one accession (WR813; *O. meridionalis*). All the accessions produced yellow colored anthers. Three variants of stigma color were observed namely, purple in 64.4% accessions, the most frequent type, white in 27.8% and light purple in 7.8% of the accessions of *Oryza* species (Figures 2k, 3p-r).

Based on seed shattering pattern, most of the accessions displayed very high seed shattering (52.2%) followed by high (16.7%) and moderate (14.4%) (Figure 2l). The low (7.8%) and very low (8.9%) seed shattering observed mainly in cultivated rice accessions. After harvest, the threshability pattern of wild rice accessions was assessed by hand threshing. In most of the accessions, we observed easy threshability (44.4%) followed by loose (24.4%) and intermediate type (16.7%) (Figure 2m). The difficult (8.9%) and moderately difficult type (5.6%) of threshability were also found. Hull color observed at pre-maturity stage, showed a wide range of phenotypic variation (Figure 4). Green color recorded in 40.0% accessions, was the most frequent color followed by brown (32.2%), brown furrows on green (15.6%) and brown spots on green (7.8%) (Figure 2n). However, green striped white (3.3%) in WR746, WR767 (*O. nivara*) and WR815 (*O. glumaepatula)* and black (1.1%) found in WR813 (*O. meridionalis*). For lemma and palea pubescence, short hairs type (72.2%) was the prominent followed by hairs on upper portion (26.7%) and glabrous (1.1%) observed in WR806 (*O. glaberrima*). Pericarp color was recorded after dehusking rice grains (Figure 5a-e). Five pericarp colors observed based on the visual observation. Most of the accessions showed light brown (44.4%), followed by brown (24.4%), speckled brown (16.7%), white (8.9%) and green (5.6%) colored pericarp (Figure 2o).

Genetic Diversity in Wild Rice Germplasm

The Shannon-Weiner diversity index and Pielou's measure of evenness showed enormous variation within and between species for morpho-qualitative traits in *Oryza* germplasm accessions (Table 3). However, anthers displayed different shades of yellow colors, light yellow in immature to dark yellow in matured anthers with zero values of diversity index and evenness. The high level of diversity recorded in 11 morpho-qualitative traits namely, awn color, culm angle, panicle type, lemma and palea color, threshability, pericarp color, seed shattering, panicle exsertion, flag-leaf angle, basal-leaf sheath color and apiculus color. Six traits *viz.*, ligule shape, stigma color, auricle color, lemma and palea pubescence, ligule color and leaf-blade pubescence showed

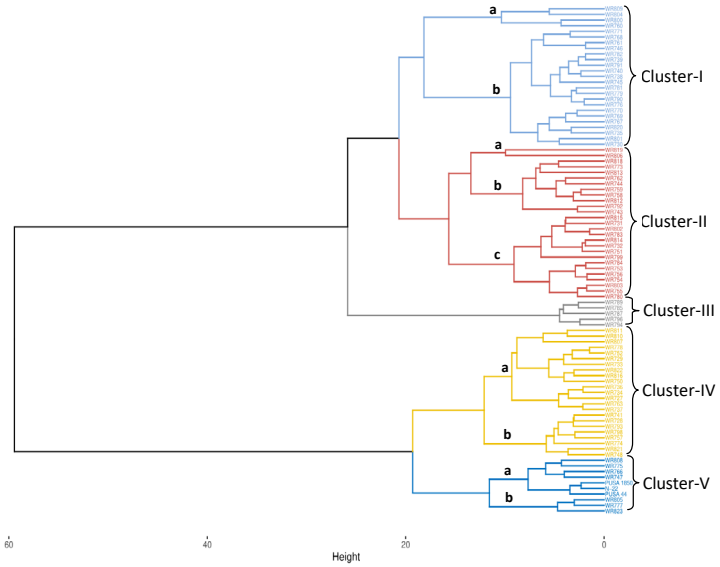


Figure 6: Dendrogram constructed using Ward's distance matrices based on data of 20 morpho-qualitative traits recorded in 90 accessions of *Oryza* species. Five clusters are shown on the right margin of the dendrogram with cluster-I divided into two subclusters (a-b), cluster-II into three subclusters (a-c), and cluster-IV and cluster-V each into two subclusters (a-b).

Table 3: Shannon -Weiner diversity index and evenness of 90 *Oryza* accessions based on qualitative data

Sl. No.	*Traits*	*Evenness (J)*			*Shannon-Weiner index (H')*		
		Between species	*Within nivara*	*Within rufipogon*	*Between species*	*Within nivara*	*Within rufipogon*
1.	Growth habit	0.31	0.00	0.66	0.22	0.00	0.66
2.	Culm angle	0.87	0.94	0.83	1.39	1.30	1.33
3.	Basal-leaf sheath color	0.85	0.95	0.83	1.18	1.32	1.15
4.	Flag-leaf angle	0.88	0.89	0.70	1.22	1.24	0.97
5.	Leaf blade pubescence	0.72	0.80	0.36	0.50	0.56	0.25w
6.	Ligule color	0.37	0.41	0.36	0.52	0.45	0.25
7.	Ligule shape	0.62	0.71	0.36	0.99	0.78	0.25
8.	Auricle color	0.40	0.35	0.52	0.65	0.49	0.52
9.	Panicle exsertion	0.78	0.86	0.83	1.26	1.20	0.83
10.	Panicle type	0.86	0.82	0.93	1.39	1.32	0.93
11.	Awning	0.57	0.17	0.58	0.39	0.12	0.58
12.	Awn color	0.87	0.80	0.63	1.40	1.29	0.63
13.	Apiculus color	0.68	0.82	0.68	1.09	1.13	0.68
14.	Stigma color	0.76	0.81	0.66	0.84	0.89	0.66
15.	Anther color	0.00	0.00	0.00	0.00	0.00	0.00
16.	Lemma and palea color	0.77	0.91	0.83	1.38	1.46	0.83
17.	Seed shattering	0.83	0.75	0.79	1.33	1.04	0.79
18.	Threshability	0.86	0.79	0.88	1.38	1.10	0.88
19.	Pericarp color	0.86	0.79	0.88	1.38	1.10	0.83
20.	Lemma and palea pubescence	0.58	0.90	0.85	0.64	0.63	0.85
	Average	0.67	0.67	0.66	0.96	0.87	0.69

Table 4: Distribution of 90 accessions of *Oryza* species into different clusters based on Wards' distance dendrogram

luster I (25 accessions)		*Cluster II (27 accessions)*		*Cluster III (5 accessions)*	*Cluster IV (23 accessions)*		*Cluster V (10 accessions)*
WR809	WR738	WR819	WR802	WR789	WR811	WR734	WR808
WR804	WR745	WR806	WR783	WR785	WR810	WR727	WR775
WR800	WR781	WR818	WR814	WR787	WR807	WR763	WR766
WR760	WR779	WR773	WR732	WR796	WR778	WR737	WR747
WR771	WR790	WR813	WR751	WR794	WR752	WR741	PUSA1850
WR768	WR776	WR762	WR799		WR729	WR728	N22
WR761	WR770	WR744	WR784		WR733	WR793	PUSA44
WR746	WR769	WR759	WR753		WR822	WR798	WR805
WR782	WR767	WR758	WR756		WR816	WR757	WR777
WR739	WR820	WR812	WR754		WR750	WR774	WR823
WR791	WR735	WR792	WR803		WR736	WR821	
WR740	WR801	WR743	WR755			WR748	
	WR730	WR815	WR780				
		WR731					

moderate level of morphological diversity, whereas two traits like growth habit and awning displayed low diversity amongst 90 accessions of 10 *Oryza* species. The maximum evenness observed in the traits *viz*., flag leaf angle/attitude (0.88), awn color (0.87) and culm angle (0.87). The minimum evenness found in growth habit (0.31) followed by ligule color (0.37).

We observed within species high variability in *O. nivara* accessions for 11 traits namely, lemma and palea color, basal-leaf sheath color, panicle type, culm angle, awn color, flag-leaf angle, panicle exsertion, apiculus color, threshability, pericarp color and seed shattering. The moderate within species variability observed for stigma color, ligule shape, leaf-blade pubescence, and lemma and palea pubescence,

while it was low for auricle color, ligule color and awning. However, there was no variation for two qualitative traits *viz*., growth habit and anther color for 41 *O. nivara* accessions. For *O. nivara* accessions, high evenness observed for 11 traits *viz*., basal leaf sheath color, culm angle, lemma and palea color, lemma and palea pubescence, flag-leaf angle, panicle exsertion, panicle type, apiculus color, stigma color, awn color and leaf-blade pubescence. Low evenness observed in auricle color, ligule color and awning.

Within species high diversity observed in *O. rufipogon* accessions for two traits *viz*., culm angle and basal-leaf sheath color, while the moderate level of variation found for 14 traits namely, flag-leaf angle, panicle type, threshability, lemma and palea pubescence, panicle exsertion, pericarp color, lemma and palea color, seed shattering, apiculus color, stigma color, growth habit, awning, auricle color and awn color. However, traits such as ligule shape, ligule color and leaf-blade pubescence revealed low within species diversity. The maximum evenness observed in panicle type (0.93) followed by threshability (0.88) and pericarp color (0.88). The minimum evenness was observed for ligule shape, ligule color and leaf- blade pubescence.

Genetic Inter-relationships in Wild Rice Germplasm

The dendrogram based on Ward's distance grouped the 90 accessions into five major clusters (Figure 6). Cluster-I included 25 accessions, of which 14 accessions belonged to *O. nivara*, 9 to *O. rufipogon*, one to *O. glaberrima* and one to *O. sativa* var. *spontanea* (Table 4). Cluster-II grouped 27 accessions consisted of 12 of *O. nivara*, 8 of *O. rufipogon*, two accessions each of *O. officinalis*, *O. meridionalis* and *O. glumaepatula*, and one accession of *O. glaberrima*. Cluster-III contained five accessions of *O. rufipogon* only. Cluster-IV separated 23 accessions consisting of 13 of *O. nivara*, four of *O. rufipogon*, two of *O. glaberrima* and one accession each of *O. meridionalis*, *O. barthii*, *O. sativa* var. *spontanea* and *O. longistaminata*. Cluster-V grouped 10 accessions, two accessions of *O. nivara*, three of *O. rufipogon*, three of *O. sativa* (checks), and one accession each of *O. glaberrima* and *O. longistaminata*. In four clusters, 85 accessions of nine *Oryza* species were grouped, each cluster containing accessions of three or more species because of shared similarities in morphological traits between accessions of different species. The cluster-II grouped the maximum accessions (27) of six different species including two accessions of CC-genome species, *O. officinalis* and was divided into three sub-clusters (a-c). Similarly, cluster-I the second largest cluster included 25 accessions of four species mainly *O. nivara* and *O. rufipogon* (23 accessions), and cluster-IV grouped 23 accessions of seven species of AA-genome. The second smallest cluster, cluster-V separated 10 accessions of five species. Thus, the 19 morpho-qualitative traits, which revealed phenotypic variations, shared the similarities amongst eight different species of AA-genome of genus *Oryza*.

Discussion

The utilization of genetic diversity from wild genetic resources is crucial for rice improvement. Accurate phenotypic characterization and organization of diversity would help to determine breeding strategies and facilitate appropriate choices for germplasm conservation (Khush and Virk, 2000). Traditionally, the identification and classification of species have been done mainly through morphological characterization (Vaughan and Morishima, 2003). The qualitative traits have been used for identification, characterization and classification of wild species germplasm, landraces and varieties of rice because these traits are stable and express in all environmental conditions (Singh *et al*., 2018; Tiwari *et al*., 2020; Sathishkumar *et al*., 2021). However, characterization of species diversity could help to decode their dynamic relationship and identification of suitable breeding material for rice improvement (Samal *et al*., 2018; Brar and Khush, 2018). Awning, seed shattering and dormancy are the key characteristics present in rice CWRs (Vaughan *et al*., 2008; Sun and Xu, 2021; Ishikawa *et al*., 2022). The anthocyanin pigmentation traits namely, basal leaf-sheath color, ligule color, auricle color, awn color, apiculus color, stigma color, anther color, hull color and pericarp color could distinguish cultivated species from wild rice species for classification and identification of species (Vaughan *et al*., 2003; Sun *et al*., 2018). The aim of the present study was to harness the genetic variation in morpho-qualitative traits available within and between the crop wild relatives of AA-genome species of rice.

In India, CWRs of rice occur mainly in temperate regions of Eastern and Western-Himalayas, sub-tropical regions of Indo-Gangetic-Brahmaputra plains, Central and Western parts, and tropical peninsular regions (Sharma and Patra, 2010). The annual wild progenitor (*O. nivara*) of Asian cultivated rice (*O. sativa*) occurs in seasonal pools, edges of ponds and tanks, ditches and swampy areas (Singh *et al*., 2018; Tiwari *et al*., 2020). It also grows sympatrically with *O. rufipogon* (perennial wild progenitor of *O. sativa*) and naturally hybridizes with both *O. sativa* and *O. rufipogon* forming *nivara-sativa-rufipogon* complex. These natural hybrids and their introgressed hybrids are commonly referred as *O. sativa* f. *spontanea* (Sharma, 2003; Sweeney and McCouch, 2007). We recorded data on 20 qualitative traits in 88 accessions of *Oryza* AA-genome species, of which polymorphism observed in 19 traits except for anther color. The morpho-qualitative traits *viz*., flag-leaf attitude, panicle type, panicle exsertion, awning, seed shattering, and panicle threshability are analysed mainly for genetic diversity assessment. However, traits namely, growth habit, culm angle, basal leaf-sheath color, leaf-blade pubescence,

ligule shape and color, auricle color, awn color, stigma color, anther color, apiculus color, lemma-palea color and pubescence, and pericarp color are normally utilized for taxonomic and classification purposes (Vaughan, 1994). These traits varied between accessions of the same species (intra-species) and of different species (inter-species) in the present study. Our results corroborate with the findings on qualitative trait studied in wild rice species of AA-genome by earlier researchers in India (Singh *et al*., 2018; Samal *et al*., 2018; Tiwari *et al*., 2020) and abroad (Zamora *et al*., 2003, Arrieta-Espinoza *et al*., 2005; Dong *et al*. 2010).

In the present study, the differentiating traits among wild species were culm angle, basal-leaf sheath color, flag-leaf angle, panicle types and exsertion, ligule shape, awning, awn color and seed shattering. The accessions of *O. nivara* possessed annual growth habit and displayed moderate to high level of within species variation for 18 qualitative traits. In *O. nivara*, the prominent traits were semi-erect/erect culm angle (64.8%), green/light purple basal-leaf sheaths (68.2%), erect/intermediate flag-leaf attitude (61.0%), white ligule color (87.8%), acuminate ligule shape (65.8%), green auricles (87.8%), just exserted panicles (51.2%), red awns (46.3%), high to very high seed shattering (80.9%), purple stigma (63.4%) and light brown to brown pericarp (80.4%). Similarly, *O. rufipogon* accessions expressed annual to perennial growth habit, semi-erect to procumbent (89.6%), green to light purple basal leaf-sheath color (79.3%), horizontal flag-leaf attitude (65.5%), white ligules (93.1%), 2-cleft ligule shape (93.1%), green auricles (82.75), well-exserted panicles (44.8%), spreading/ horizontal panicle type (68.9%), red awns (68.9%), purple stigma (68.9%), high to very seed shattering (72.4%) and light brown to brown pericarp color (62.0%). Similar observations on morpho-qualitative traits were also made by Samal *et al*. (2018) after studying a large collection of *O. nivara* and *O. rufipogon* from Odisha and West Bengal. They reported a high level of morphological variation in both *O. nivara* and *O. rufipogon* accessions. The high variability was detected for basal-leaf color, leaf angle, culm attitude, internode color and flag leaf attitude in both Asian wild rice species of AA genome. In *O. nivara*, green basal-leaf sheath was predominant (52%), while majority (47%) of *O. rufipogon* accessions produced purple basal-leaf sheath. The present study revealed the existence of higher variation in *O. nivara* (H′ = 0.87) than *O. rufipogon* (H′ = 0.69) may be due to larger sample size of *O. nivara* (n=41) than *O. rufipogon* (n=29). The accessions of *O. sativa* f. *spontanea* were identified having erect culm, green basal-leaf sheath color; erect panicle type, purple stigma with brown pericarp (Samal *et al*., 2018). The dendrogram based on morphological traits clearly separated *O. nivara* and *O. rufipogon* accessions into two different classes. Singh *et al*. (2018) studied a large germplasm of 418 accessions of CWRs collected from diversity hotspot regions of India and reported tremendous variability for awning, awn color, leaf-blade color, basal-leaf color, leaf angle, growth habit, culm angle, panicle type and panicle exsertion. They also emphasized that the *O. nivara* was predominant morpho-taxonomic species across India.

In a study of Srilankan wild rices, Sandamal *et al*. (2021) reported that the *O. nivara* accessions were identified by semi-erect to spreading culm angle, green basal-leaf sheath color, intermediate flag-leaf attitude, acuminate ligule shape, low to high seed shattering; while, *O. rufipogon* accessions were identified with procumbent culm angle, horizontal flag leaf attitude, purple basal-leaf sheath color, 2-cleft ligule shape, purple stigma, open panicle type and red awns. The Asian cultivated rice (*O. sativa*) and African cultivated rice (*O. glaberrima*) produced non-shattering, awnless, erect culm angle and flag-leaf attitude, white stigma color and white pericarp color phenotypes, which clearly distinguished these cultivated species from wild species of *Oryza* AA genome. In our dendrogram, 85 accessions of nine *Oryza* species grouped in four clusters with each including accessions of three to seven species of AA genome because of shared similarities in morphological traits by *O. sativa* species complex.

Conclusion

Huge morphological variability detected for 19 morpho-qualitative traits in 88 germplasm accessions of CWRs of rice consisting of eight species of AA-genome. Shannon-Weiner diversity index revealed both within and between species variability for all traits except anther color. Within species variability analysis revealed the presence of higher diversity in *O. nivara* than the *O. rufipogon* for most of the traits. Cluster analysis grouped the accessions of CWRs into five major clusters with each cluster possessing accessions of multiple species showing morphological similarity between accessions of different species *O. sativa* species complex. The vast phenotypic variability revealed in the accessions of the progenitor species of cultivated Asian rice could be utilized for genetic improvement programmes of rice.

Acknowledgement

First author is grateful to the Director, ICAR-IARI, New Delhi for granting Senior Research Fellowship during Ph.D. study. Authors are thankful to Director, ICAR-National Bureau of Plant Genetic Resources, New Delhi for providing research facilities, and Head, Division of Germplasm Conservation and the Curator of National Gene Bank, ICAR-NBPGR, New Delhi for providing the seeds of various accessions of *Oryza* species.

References

Alsantely, A., Gutaker, R., Navarrete Rodríguez, M. E., Arrieta-Espinoza, G., Fuchs, E. J., Costa de Oliveira, A., Tohme, J., Zuccolo, A., Wing R. A., & Fornasiero, A. (2023). The International *Oryza* Map Alignment Project (IO MAP): the Americas - Past achievements and future directions. *J. Exp.*

Bot., **74(5)**: 1331-1342.

Arrieta-Espinoza, G., Sanchez, E., Vargas, S., Lobo, J., Quesada, T., & Espinoza, A. M. (2005). The weedy rice complex in Costa Rica. I. morphological study of relationships between commercial rice varieties, wild *Oryza* relatives and weedy types. *Genet. Resour. Crop Evol.*, **52(5)**: 575-587.

Bioversity International, IRRI, & WARDA (2007). *Descriptors for Wild and Cultivated Rice* (*Oryza* spp.), Rome, Italy; International Rice Research Institute, Los Banos, Philippines; WARDA, Africa Rice Center, Cotonou, Benin, pp. 22-42.

Brar, D. S., & Khush, G. S. (2018). Wild relatives of rice: a valuable genetic resource for genomics and breeding research. In: Mondal, T., Henry, R. (eds.) *The Wild Oryza Genomes*. *Compendium of Plant Genomes*. Springer, Cham. pp. 1-25.

Dong, Y., Pie, X., Yuan, Q., Wu, H., Wang, X., Jia, S., & Peng, Y. (2010). Ecological, morphological and genetic diversity in *Oryza rufipogon* Griff. (Poaceae) from Hainan Island, China. *Genet. Resour. Crop Evol.*, **57(6)**: 915-926**.**

FAO (2022). *World Food and Agriculture – Statistical Yearbook 2022*. Rome. *https://doi.org/10.4060/ cc2211en*.

Fornasiero, A., Wing, R. A., & Ronald, P. (2022). Rice domestication. *Curr. Biol.*, **32(1)**: 20-24.

Gaikwad, K. B., Singh, N., Kaur, P., Rani, S., Babu H, P., & Singh, K. (2020). Deployment of wild relatives for genetic improvement in rice (*Oryza sativa*). *Plant Breed.*, **140(1)**: 23-52.

Harlan, J. R., & de Wet, J. M. (1971). Toward a rational classification of cultivated plants. *Taxon*, **20(4)**: 509-517.

Hien, N. L., Sarhadi, W. A., Oikawa, Y., & Hirata, Y. (2007). Genetic diversity of morphological responses and the relationships among Asia aromatic rice (*Oryza sativa L.*) cultivars. *Tropics*, **16(4)**: 343-355.

Hijmans, R. J., Guarino, L., Cruz, M., & Rojas, E. (2001). Computer tools for spatial analysis of plant genetic resources data: 1. DIVA-GIS. *Plant Genetic Resources Newsletter*, **127**: 15-19.

International Rice Research Institute (2013). *Standard Evaluation System for Rice*, 5th Edition. Los Banos, Philippines, p.55.

Ishikawa, R., Castillo, C. C., Htun, T. M., Numaguchi, K., Inoue, K., Oka, Y., Ogasawara, M., Sugiyama, S., Takama, N., Orn, C., Inoue, C., Nonomura, K. I., Allaby, R., Fuller, D. Q., & Ishii, T. (2022). A stepwise route to domesticate rice by controlling seed shattering and panicle shape. *Proceedings of the National Academy of Sciences*, *USA*, **119(26)**: e2121692119.

Joseph, J., Francies, R. M., Zachariah, G., & Santoshkumar, A. V. (2007). Characterisation of Navara (*Oryza sativa* L.) - a traditional medicinal rice of Kerala for qualitative traits. *Indian J. Agric. Res.*, **41(4)**: 267-271.

Khan, A., Ahmad, H., Shuaib, M., Bahadur, S., & Hussain, F. (2020). Enumeration of genetic diversity of wild rice through phenotypic trait analysis. *Gene Reports*, **21**, 100797.

Kharel, R., Subedi, S., Ghimire, D., & Shrestha, S. (2022). Characterization of Nepalese rice (Oryza sativa L.) landraces for qualitative traits. *J Agric Nat Resour.*, **5(1)**: 40-51.

Khush, G. S., & Virk, P. S. (2000). Rice breeding: achievements and future strategies. *Crop Improv*ement **27**: 115-144.

Khush, G. S. (2013). Strategies for increasing the yield potential of cereals: case of rice as an example. *Plant Breed.*, **132(5)**: 433-436.

Le, S., Josse, J., & Husson, F. (2008). FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software*, **25**: 1-18.

Lei, Q., Zhou, J., Zhang, W., Luo, J., Wu, K., & Long, C. (2018). Morphological diversity of panicle traits in Kam fragrant glutinous rice (*Oryza sativa*). *Genet. Resour. Crop Evol.*, **65**: 775-786.

McCouch, S. R., Baute, G. J., Bramel, P., Bretting, P. K., Buckler, E., *et al.* (2013). Feeding the future. *Nature,* **499**: 23-24.

McCouch, S. R., McNally, K, L., Wang, W., & Hamilton, R. S. (2012). Genomics of gene banks: A case study in rice. *Am. J. Bot.*, **99**: 407-423.

Pielou, E. C. (1966). The measurement of diversity in different types of biological collections. *J. Theor. Biol.*, **13**: 131-144.

Rabara, R. C., Ferrer, M. C., Diaz, C. L., Newingham, M. C. V., & Romero, G. O. (2014). Phenotypic diversity of farmers' traditional rice varieties in the Philippines. *Agron.*, **4(2)**: 217-242.

Rao, N. N. D., Roja, V., Tushara, M., Rao, V. S., & Rao, V. S. (2021). Characterization and Diversity Analysis of Rice Germplasm. *BFAIJ*, **13(3a)**: 170-179.

Samal, R., Roy, P. S., Sahoo, A., Kar, M. K., Patra, B. C., Marndi, B. C., & Gundimeda, J. N. R. (2018). Morphological and molecular dissection of wild rices from eastern India suggests distinct speciation between *O. rufipogon* and *O. nivara* populations. *Sci. Rep.*, **8(1)**: 2773.

Sanchez, P. L., Wing, R. A., & Brar, D. S. (2013). The Wild Relative of Rice: Genomes and Genomics. In: Zhang, Q. and Wing, R. A. (eds.), *Genetics and Genomics of Rice*, *Plant Genetics and Genomics*. Springer, New York, pp. 9-25.

Sandamal, S., Tennakoon, A., Amarasekera, D. A. B. N., Marambe, B., & Ratnasekera, D. (2021). Functional trait diversity of wild rice species in Sri Lanka: implications for field identification and application. *J Natl. Sci. Found*. **49(3)**: 369-382.

Sathishkumar, P., Kumar, P. R., Maddala, S. S. R. S. C., Rao, S. D., Sundaram, R. M., Gopala K. S., Singh, A. K., Singh, K., Rao, S. L. V., Rani, S. N., & Madhav, S. M. (2021). Development of core set of aromatic rice (*Oryza sativa* L. *indica*) based on molecular and morphological diversity. *Genet. Resour. Crop Evol.,* **68(2)**: 441-450.

Shannon, C. E., & Weaver, W. (1949). *The Mathematical Theory of Communication*. University of Illinois Press, Urbana, USA.

Sharma, S. D. & Patra, B. C. (2010). Exploration, collection, conservation and utilization of wild rices for the improvement of cultivated rice. In: Sharma, S. D. (ed.), *Genetic Resources of Rice in India–Past and Present*. Todays and Tomorrow's Printers and Publishers, New Delhi, pp. 323-346.

Sharma, S.D. (2003). Species of Genus *Oryza* and their Interrelationships. In: Nanda, J. S. and Sharma, S. D., (eds.), *Monograph on Genus Oryza*. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, pp. 73-111.

Singh, B., Singh, N., Mishra, S., Tripathi, K., Singh, B. P., Rai, V., Singh, A. K., & Singh, N. K. (2018). Morphological and molecular data reveal three distinct populations of Indian wild rice *Oryza rufipogon* Griff. species complex. *Front. Plant Sci.*, **9**: 123. *Doi: 10.3389/fpls.2018.00123*.

Sow, M., Sido, A., Laing, M., & Ndjiondjop, M. N. (2014). Agro-morphological variability of rice species collected from Niger. *Plant genet. resour.*, **12(1)**: 22-34.

Stein, J. C., Yu, Y., Copetti, D., Zwickl, D.J., Zhang, L., Zhang, C., Chougule, K., Gao, D., Iwata, A., Goicoechea, J.L., & Wei, S. (2018). Genomes of 13 domesticated and wild rice relatives highlight genetic conservation, turnover and innovation across the genus *Oryza*. *Nat. Genet.,* **50(2)**: 285–296.

Sun, X., Zhang, Z., Chen, C., Wu, W., Ren, N., Jiang, C., Yu, J., Zhao,

Y., Zheng, X., Yang, Q., Zhang, H., Li, J., & Li, Z. (2018). The C–S–A gene system regulates hull pigmentation and reveals evolution of anthocyanin biosynthesis pathway in rice. *J. Exp. Bot.*, **69(7)**: 1485-1498.

Sweeney, M., & McCouch, S. (2007). The complex history of the domestication of rice. *Annals of Botany*, **100(5)**: 951-957.

Tiwari, S., Yadav, M. C., Dikshit, N., Yadav, V. K., Pani, D. R., & Latha, M. (2020). Morphological characterization and genetic identity of crop wild relatives of rice (*Oryza sativa* L.) collected from different ecological niches of India. *Genet. Resour. Crop Evol.*, **67**: 2037-2055.

Vaughan, D. A. (1989). The genus *Oryza* L. Current Status of Taxonomy. In: *IRRI Research Paper Series,* Manila, IRRI, Philippines. pp. 1–21.

Vaughan, D. A. (1994). *The Wild Relatives of Rice: A Genetic Resources Handbook*. International Rice Research Institute, Las Banos, Philippines.

Vaughan, D. A., Kadowaki, K. I., Kaga, A., & Tomooka, N. (2005). On the phylogeny and biogeography of the genus *Oryza*. *Breed. Sci.*, **55(2)**: 113-122.

Vaughan, D. A., Lu, B. R., & Tomooka, N. (2008). The evolving story of rice evolution. *Plant Sci.*, **174(4)**: 394-408.

Vaughan, D. A., Morishima, H., & Kadowaki, K. (2003). Diversity in the *Oryza* genus. *Curr. Opin. Plant Biol.*, **6(2)**: 139-146.

Wing, R. A., Purugganan, M. D., & Zhang, Q. (2018). The rice genome revolution: from an ancient grain to Green Super Rice. *Nat. Rev. Genet.*, **19(8)**: 505-517.

Xu, R., & Sun, C. (2021). What happened during domestication of wild to cultivated rice. *The Crop Journal*, **9(3)**: 564-576.

Zamora, A., Barboza, C., Lobo, J., & Espinoza, A. M. (2003). Diversity of native rice (*Oryza*: Poaceae) species of Costa Rica. *Genet. Resour. Crop Evol.,* **50(8)**: 855-870.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.10

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Circa situm Conservation of Tree Genetic Resources: A Case Study from the Central Western Ghats of Karnataka, India

Gudihalli M. Puneeth[1], Ravi Gowthami[2], Ramesh Vasudeva[3], Kerekoppa M. Laxmisha[1] and Sunil Archak[2*]

Abstract

Tree species are an integral part of agrobiodiversity. Several tree species have multifarious uses. India's Western Ghats, with diverse climate, topography and soils, are home to a number of tree species. A study was conducted in the adjoining areas of Karnataka's Central Western Ghats, including coastal lowlands/plains (Dakshina Kannada) and high mountain ranges (Shivamogga and Uttara Kannada). A total of 93 species belonging to 75 genera and 41 families are being conserved *circa situm* by the farmers of this region. From our study, it was found that the conservation of tree species solely depends on the farmers' perception of the utilization of these species *i.e.,* "conservation through use" and the species recorded in the present study area are conserved due to their uses as timber, fruit/nut/spice/ornamental, border/windbreaker, fuel, cultural significance, shade and other uses (gum, resin, soil conservation, etc.). Conservation through *circa situm* recognizes the ownership of the farmers of a given region and also considers the socio-economic context of conservation by meeting the food, nutrition, livelihood and income security of the farmers.

Keywords: *Circa situm*, Conservation, Tree genetic resources, Conservation through use, Western Ghats.

[1]Division of Plant Genetic Resources, ICAR-Indian Agricultural Research Institute, Pusa Campus, New Delhi, India

[2]ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India

[3]College of Forestry (Sirsi), UAS Dharwad, Karnataka, India

***Author for correspondence:**

sunil.archak@icar.gov.in

Received: 11/05/2023 **Revised:** 27/07/2023

Accepted: 27/07/2023

How to cite this article: Puneeth, G.M., Gowthami, R., Vasudevak, R., Laxmisha, K.M. and Archak, S. (2023). *Circa situm* Conservation of Tree Genetic Resources: A Case Study from the Central Western Ghats of Karnataka, India. Indian J. Plant Genetic Resources. 36(3), 415-421.

DOI: 10.61949/0976-1926.2023.v36i03.10

Introduction

India has rich diversity in terms of climate, weather, topography and culture, which has resulted in a vast diversity in flora and fauna, and is one of the 12 megacentres of the world (Gowthami *et al.*, 2021). Trees are an integral part of biodiversity and have been meeting multipurpose needs *viz.,* enhancing agrobiodiversity, mitigating the greenhouse effect, meeting the needs of humans (medicine, food, timber, fuel, fibres, ornamental, cultural and spiritual purposes) (Dhyani *et al.*, 2022). A recent assessment of the number of trees on earth has confirmed the existence of ~73,000 tree species, among which ~9,000 are yet to be discovered (Cazzolla Gatti *et al.*, 2022). Among these, 20.34% (14,853) species are threatened [critically endangered (CR)- 2,931; endangered (EN)- 5,890 and vulnerable (VU)- 6,032,54 are extinct (EX) and 28 species are already extinct in the wild (EW)] (IUCN, 2023). In India, about 2603 tree species are reported, of which 24.97% (650 species) are endemic and nearly 18% (469) tree species are threatened with extinction (Dhyani *et al.*, 2022). This may be due to overexploitation for timber and other products from the natural habitat, habitat loss naturally and human-induced, forest clearance, disease and climate change (Gowthami *et al.*, 2021; Dhyani *et al.*, 2022).

Karnataka state is endowed with a diverse climate, topography, soils and which has resulted in rich biodiversity and reported to have the highest number of tree species (325) followed by Tamil Nadu (252), Andhra Pradesh (242) and Kerala (238) as reported in the

current India State of Forest Report (FSI, 2019). In Karnataka, a total of 146 tree species are reported to be under different levels of threat; of which 75 tree species are threatened (CR-6; EN- 28; VU- 41), 54 are extinct (EX), 28 species are already extinct in wild (EW), 17 are near threatened, 48 are least concern and 6 are data deficient (IUCN, 2023). The Western Ghats of India are nearly 1,600 km long range of mountains from Tapti river in North to Kanyakumari in South, also recognized as one of the world's biodiversity hot spot rich in flora and fauna diversity. The southern Western Ghats covered in Karnataka, Kerala and Tamil Nadu has rich diversity *i.e.,* out of the 4000 species of flowering plants estimated, 3,900 occur in the region (Sasidharan, 2003). Western Ghats of Karnataka cover Belagavi, Chamarajanagara, Chikkamagaluru, Dakshina Kannada, Hassan, Kodagu, Mysuru, Shivamogga, Udupi and Uttara Kannada districts of the state. A great diversity of tree species having great economic importance exists in this region. Since the beginning, these tree resources are an essential component meeting the several demands of the communities *viz.,* five F's of food, fiber, forests, flowers, and fuel, also meeting the additional necessities such as shelter and medicine, ultimately meeting the food, nutrition and livelihood security, directly and indirectly balancing the biodiversity. The importance of conserving these tree genetic resources has been in place for many years and the government, research institutes, and non-government organizations have made efforts to safely conserve trees *in-situ* and *ex-situ* methods.

Generally, conservation in seed banks is the main approach for *ex-situ* conservation. However, seed conservation of many tree species is challenging due to the recalcitrant seed behavior, long juvenile phases, and highly heterozygous nature and therefore do not breed true, thus mainly propagated by cuttings/grafts. As a result, many of the species are maintained as living collects in orchards/ field genebanks/forest genebanks etc. and as *in-vitro* cultures in the *in vitro* genebanks. Among the different approaches, on-farm conservation of tree species *in-situ* in home gardens *i.e., circa situm* is one of the conservation strategies the farmers are using. *Circa situm* conservation is a form of farmer-based conservation in altered agricultural landscapes such as agroforestry, home gardens and orchards that are outside the natural habitats but within the native geographical range of a species (Agrawal *et al*., 2023; Boshier *et al*., 2004; Dawson *et al*., 2013; Vasudeva, 2022) mostly for the conservation through use. Hence, in the present study, efforts have been made to assess the level and quantum of the diversity of tree species conserved through *Circa situm* approach in the central Western Ghats of Karnataka.

Methodology

The present study was conducted in coastal lowlands/ plains (Dakshina Kannada district), high mountain ranges (Shivamogga, Uttara Kannada districts) of the central Western Ghats, Karnataka, India during November 2020 to 2021. Data was documented from the different custodian farmers using a questionnaire and semi-structured interviews. Initially, the socio-economic survey was conducted to document the data on different species of trees conserved. For the socio-economic surveys, semi-structured interviews were carried out with members of five farm holds in each category of small (< 2 ha), medium (2–10 ha) and large (>10 ha) farm holds in each district selected randomly. Each farm family was asked the use for growing of each species and was also asked to rank the species based on the usage.

Results and Discussion

Tree species are one of the important components of terrestrial ecosystems and agrobiodiversity. Great diversity by their direct and indirect value exists in tree species *i.e.,* they are deciduous, evergreen, ornamental, fragrant, edible fruit bearing, medicinal, timber yielding, fodder yielding, nitrogen-fixing, shade bearing, fuel yielding, dye yielding etc (Seth, 2003). Due to the multifarious uses of trees, globally, nearly 8,000 tree species are used by humans (FAO, 2014) and ~ 20.34% of tree species are threatened due to over-exploitation, deforestation, land use change, and climate change (Van Zonneveld *et al*., 2018). Conventionally, tree genetic resources are conserved *in-situ* in their natural habitat in the forests and mainly important species are conserved *ex-situ* in seed genebank, field genebank, *in-vitro* genebank and cryo-genebank. Many of the tree species are not highly domesticated and are maintained in the forests, nearby forest areas and untapped areas and, therefore, require human cultivation to persist (Brush, 1991). Some are planted or left as remnants in landscapes that are otherwise cleared for agriculture (Dawson *et al*., 2013). In such cases, farmer-based *circa situm* conservation approaches are particularly valuable for the conservation of such species. *Circa situm* conservation differs from *in-situ* (conservation of natural populations) and *ex-situ* (conservation in remote locations and gene banks) by being a conservation strategy to preserve near natural populations as artificial or human maintained populations.

In *circa situm* conservation, species are maintained within their natural ranges and climatic zones, but in habitats different from those in which they are assumed to have spent most of their evolutionary history. Otherwise, refers to the conservation of planted or remnant trees in farmlands or forest patches where natural forests or woodlands containing the same trees were once found; but the vegetation has been lost or modified significantly through anthropogenic intervention. The primary purpose of a farmer-based *circa situm* conservation may not be of conservation and species conserved are also may not be of immediate interest in the commercial market (Brush, 1991). Instead, they might be fulfilling many needs of the farmers such as food, fibers, medicine, live fences, and edibles among others (Dawson *et al*., 2013), more recently to provide

amenities and comfort in urban parks and streets (Han *et al*., 2020). It also acts as a "stepping stone" between forest patches and plays a significant role in gene flow (via pollen) as situated close enough to existing wild plants (Boshier *et al*., 2004; Dawson *et al*., 2013). *Circa situm* could be used to complement the role of *ex-situ* plantings as a second conservation population. Also plays an important role in maintaining insect, bird and mammal populations essential for pollination, biological pest control, and increasing crop productivity (Cristo'bal-Pe'rez *et al*., 2022).

In the study area, a total of 93 tree species belonging to 75 genera and 41 families were recorded, which are being actively conserved *circa situm* by the farmers (Table 1) (Figure 1). The number of stems/ trees conserved in each

Figure 1: *Circa situm* conservation model of tree species by the farmers of Central Western Ghats

Table 1: Tree species diversity in ***circa situm*** conservation of Central Western Ghats

Tree species	*Family*	*English name; Kannada name*
Acacia catechu (L.f.) Willd.	Fabaceae	Black catechu; Khadira
Acrocarpus fraxinifolius Wight and Arn	Fabaceae	Pink cedar; Balanji
Actinodaphne malabarica N.P.Balakr.	Lauraceae	Kambilivirinji
Adansonia digitata L.	Malvaceae	Baobab tree; Brahmamlika
Adenanthera pavonina L.	Fabaceae	Red lucky seed; Ane Golaganji
Aegle marmelos (L.) Correa	Rutaceae	Bael; Bilva
Aglaia elaeagnoidea Benth.	Meliaceae	Droopy leaf; Priyangu
Ailanthus integrifolia Lam.	Simaroubaceae	Tree of heaven; Hemmara
Alangium salviifolium (L.f.) Wangerin	Alangiaceae	Sage-leaved alangium; Ankola
Albizia amara (Roxb.) Boivin	Fabaceae	Bitter Albizia; Sujjalu mara
Albizia lebbeck (L.) Benth.	Fabaceae	Indian siris; Baage
Alstonia scholaris (L.) R.Br.	Apocynaceae	White cheesewood; Haale
Altingia excelsa Noronha	Altingiaceae	Oriental sweet gum; Rasamala
Anacardium occidentale L.	Anacardiaceae	Cashew nut; Godambi
Anogeissus latifolia (Roxb. Ex DC.) Wall. ex Guill. & Perr.	Combretaceae	Axlewood; Dindiga
Anthocephalus cadamba Miq.	Rubiaceae	Neolamarckia cadamba; Kadamba
Areca catechu L.	Arecaceae	Betel-nut Palm; Adike
Artocarpus altilis (Parkinson) Fosberg	Moraceae	Breadfruit; Divi halasu
Artocarpus lacucha Roxb. Ex Buch.-Ham.	Moraceae	Monkey fruit; Vatehuli
Artocarpus heterophyllus Lam.	Moraceae	Jackfruit; Halasu
Artocarpus hirsutus Lam.	Moraceae	Wild jackfruit; Hebbalsau
Atalantia monophylla (L.) DC.	Rutaceae	Indian atalantia; Kaadu nimbe
Azadirachta indica A. Juss.	Meliaceae	Neem; Bevu
Balanites aegyptiaca (L.) Delile	Zygophyllaceae	Desert dates; Karjura
Bauhinia malabarica Roxb.	Fabaceae	Malabar bauhinia; Basavanapada
Borassus flabellifer L.	Arecaceae	Palmyra palm; Taale mara
Boswellia carteri Birdw.	Burseraceae	Sali guggul; Guugulu mara
Buchanania lanzan Spreng.	Anacardiaceae	Chironji; Charoli
Butea monosperma (Lam.) Kuntze	Fabaceae	Flame of the forest; Palasha
Caesalpinia sappan L.	Fabaceae	Sappan wood; Sappanga
Callicarpa macrophylla Vahl	Lamiaceae	French mulberry; Ibbani

Calophyllum apetalum Blanco	Calophyllaceae	Konkan Beauty Leaf Tree; Bobbe mara
Canarium strictum Roxb.	Burseraceae	Black dammar; Raldhoop
Cassia fistula L.	Fabaceae	Golden Shower Tree; Konde mara
Ceiba pentandra (L.) Gaertn.	Malvaceae	Silk Cotton Tree ; Boorugada mara
Cinnamomum macrocarpum Hook.f.	Lauraceae	Dalchini; Tamaala
Cinnamomum malabathrum (Lam.) J.Presl	Lauraceae	Dalchini, Tejpatta
Cinnamomum sulphuratum Kurz	Lauraceae	Dalchini, Tejpatta
Cinnamomum wightii Meisn.	Lauraceae	Dalchini, Tejpatta
Citrus aurantifolia (Christm.) Swingle	Rutaceae	Key lime; Musambi
Citrus aurantium L.	Rutaceae	Sour Orange; Harale hannu
Citrus bergamia Risso	Rutaceae	Lemon; Kadu nimbu
Citrus limon (L.) Osbeck	Rutaceae	Lemon; Nimbe hannu
Citrus medica L.	Rutaceae	Citron; Kittale
Cocos nucifera L.	Arecaceae	Coconut; Tengu
Commiphora caudata Engl.	Burseraceae	Hill mango; Kondamavu
Cordia dichotoma (Ruiz & Pav.) Gurke	Boraginaceae	Indian cherry; Challe hannu
Cycas circinalis L.	Cycadaceae	Queen sago; Mandhichalu
Dalbergia sissoo Roxb. ex DC.	Fabaceae	Indian rosewood; Beete
Diospyros ebenum Koenig ex Retz.	Ebenaceae	Eboni; Abanasa
Diospyros sylvatica Roxb.	Ebenaceae	Forest ebony; Manjathuvara
Dysoxylum malabaricum Bedd. Ex C.DC.	Meliaceae	White cedar; Bili buddaliga
Elaeocarpus sphaericus (Gaertn.) K.Schum.	Elaeocarpaceae	Utrasum Bead tree; Rudraksha
Eucalyptus globulus Labill.	Myrtaceae	Eucalyptus; Nilgiri
Feronia limonia Swingle	Rutaceae	Wood apple; Belada hannu
Ficus benghalensis L.	Moraceae	Indian banyan tree; Ala mara
Ficus religiosa Forssk.	Moraceae	Peepal tree; Arali mara
Garcinia gummi-gutta (L.) N.Robson	Clusiaceae	Malabar tamarind; Uppage
Garcinia indica (Thouars) Choisy	Clusiaceae	Kokam; Purapuli
Garcinia morella (Gaertn.) Desr.	Clusiaceae	Indian Gamboge; Kadukaai puli
Grewia elastica Royle	Malvaceae	Phalsa; Chhaal
Juglans regia L.	Juglandaceae	Walnut; Akroot
Lagerstroemia microcarpa Wight	Lythraceae	Ben teak; Namdi mara
Leucaena leucocephala (Lam.) de Wit	Fabaceae	Horse tamarind; *Chiguru*
Madhuca indica J.F.Gmel.	Sapotaceae	Indian butter tree; Ippe
Mangifera indica L.	Anacardiaceae	Mango; Mavu
Mesua ferrea L.	Calophyllaceae	Indian rose-chestnut; Nagakesari,
Michelia champaca L.	Magnoliaceae	Champaka; Sampige
Mimusops elengi Bojer	Sapotaceae	Bullet Wood; Pagade mara
Moringa oleifera Lam.	Moringaceae	drumstick tree; Nuggegida
Murraya koenigii (L.) Spreng.	Rutaceae	Curry Leaf; Karibevu
Myristica dactyloides Wall.	Myristicaceae	Bitter Nutmeg; Kadu jaiphal
Myristica fragrans Houtt.	Myristicaceae	Nutmeg; Jatipatre
Myristica malabarica Lam.	Myristicaceae	Wild Nutmeg; Dodda jakai
Nyctanthes arbor-tristis L.	Oleaceae	Tree of Sadness; Harisringi
Phoenix dactylifera L.	Arecaceae	Datepalm; Kharjura
Phyllanthus emblica L.	Phyllanthaceae	Gooseberry; Bettanalli

Pongamia pinnata (L.) Merr.	Fabaceae	Indian Beech Tree; Honge mara
Prosopis cineraria (L.) Druce	Fabaceae	Khejri; Banni mara
Prunus dulcis (Mill.) Rchb.	Rosaceae	Almond; Badami
Psidium guajava L.	Myrtaceae	Guava; Seebe hannu
Pterocarpus santalinus L.f.	Fabaceae	Red sandalwood; Raktachandana
Quercus infectoria Oliv.	Fagaceae	Aleppo oak; Machikai
Rubia tinctorum L.	Rubiaceae	Common madder; Manjishta
Santalum album L.	Santalaceae	Sandalwood; Chandana
Saraca asoca (Roxb.) Willd.	Fabaceae	Ashoka; Ashoka
Shorea robusta C.F.Gaertn.	Dipterocarpaceae	Sal tree; Salada mara
Syzygium cumini (L.) Skeels	Myrtaceae	Jamun; Nerale
Tamarindus indica L.	Fabaceae	Tamarind; Hunase hannu
Tectona grandis L.f.	Lamiaceae	Teak; Tega
Terminalia arjuna (Roxb. ex DC.) Wight & Arn.	Combretaceae	Arjun tree; Nirmatti
Terminalia chebula Retz.	Combretaceae	Indian Almond tree; Kadu badami
Ziziphus jujuba Mill.	Rhamnaceae	Ber; Bogari

species was directly correlated with the number of use values of the species and its economic importance. A significant difference in the number of species conserved by the different farm categories *viz.,* small, medium and large, was observed in all three study areas. Irrespective of the district, a larger number of species was conserved *circa situm* farmers with larger land holdings (55.8–65.2) followed by those with medium holdings (38.2–52.2) and small holdings (12.6–14.4) (Figure 2). Higher diversity in the larger holdings may be due to the presence of larger landholdings with more 'corners' where trees can be grown and also the larger landholdings may focus less on optimizing total farm crop output by removing trees that compete with crops (Dhanya *et al*., 2013; Oli *et al*., 2015). Among the three survey areas, maximum number of tree species was conserved by farmers of Dakshina Kannada district (67) and Uttara Kannada (65.2) followed by Shivamogga (55.8). We also observed that species conserved by medium and small farmers were driven by their potential value of the species. Such high correlation

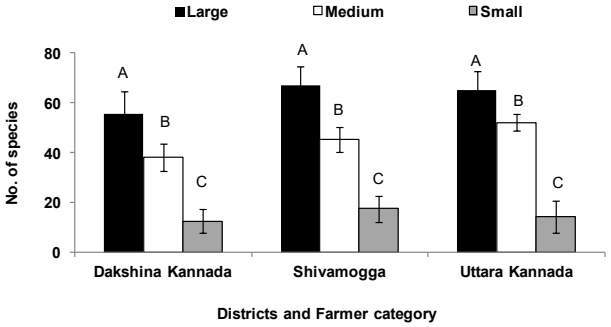


Figure 2: Mean number of tree species conserved *circa situm* by the farmers of different land holdings in central Western Ghats. Data represents mean ± SE of five farm holds in each category. Significant differences ($p \leq 0.05$) are presented by different alphabets analyzed by Duncan's Multiple Range Test.

of use values and community importance given to a species have also been documented earlier (Vasudeva *et al*., 2015).

The farmers conserve trees for their tangible and intangible benefits, termed "conservation through use". As those species enhance food, fuel and medical security, especially for low-income rural people and during hungry periods, diversify income, lower production risk and optimize the management of resources (Arnold and Dewees 1995). The farmer's preference for tree species was evaluated based on the respondents' rankings. Though most of the tree species possess multifarious uses, farmer's perception of conservation revealed that the major purpose for conservation in the study area was due to its use as timber, fruit/nut/leaves/bark, border/windbreaker, fuel, cultural significance, shade and other (gum, resin, soil conservation, etc.) (Figure 3). Among several species conserved *circa situm*, > 40% species are commercially important which are being conserved for their edible fruits or commercially important economic parts like nuts, bark, leaves etc. *Adansonia digitata*, *Aegle marmelos*, *Anacardium occidentale*, *Areca catechu*, *Artocarpus altilis*, *A. lacucha*, *A. heterophyllus*, *A. hirsutus*, *Atalantia monophylla*, *Balanites aegyptiaca*, *Borassus flabellifer*, *Buchanania lanzan, Callicarpa macrophylla, Cinnamomum macrocarpum*, *Cinnamomum malabathrum*, *C. sulphuratum*, *C. wightii*, *Citrus aurantifolia*, *C. aurantium*, *Citrus bergamia*, *C. limon*, *C. medica*, *Cocos nucifera*, *Commiphora caudate*, *Cordia dichotoma*, *Garcinia gummi-gutta*, *G. indica*, *G. morella*, *Grewia elastic*, *Juglans regia*, *Feronia limonia*, *Mangifera indica*, *Michelia champaca*, *Moringa oleifera*, *Murraya koenigii*, *Myristica dactyloides*, *M. fragrans*, *M. malabarica*, *Nyctanthes arbor-tristis*, *Phoenix dactylifera*, *Phyllanthus emblica*, *Prunus dulcis*, *Psidium guajava*, *Quercus infectoria*, *Syzygium cumini and Ziziphus jujube*. Next major group of timber purpose solely or in addition to other benefits. *Acacia catechu*,

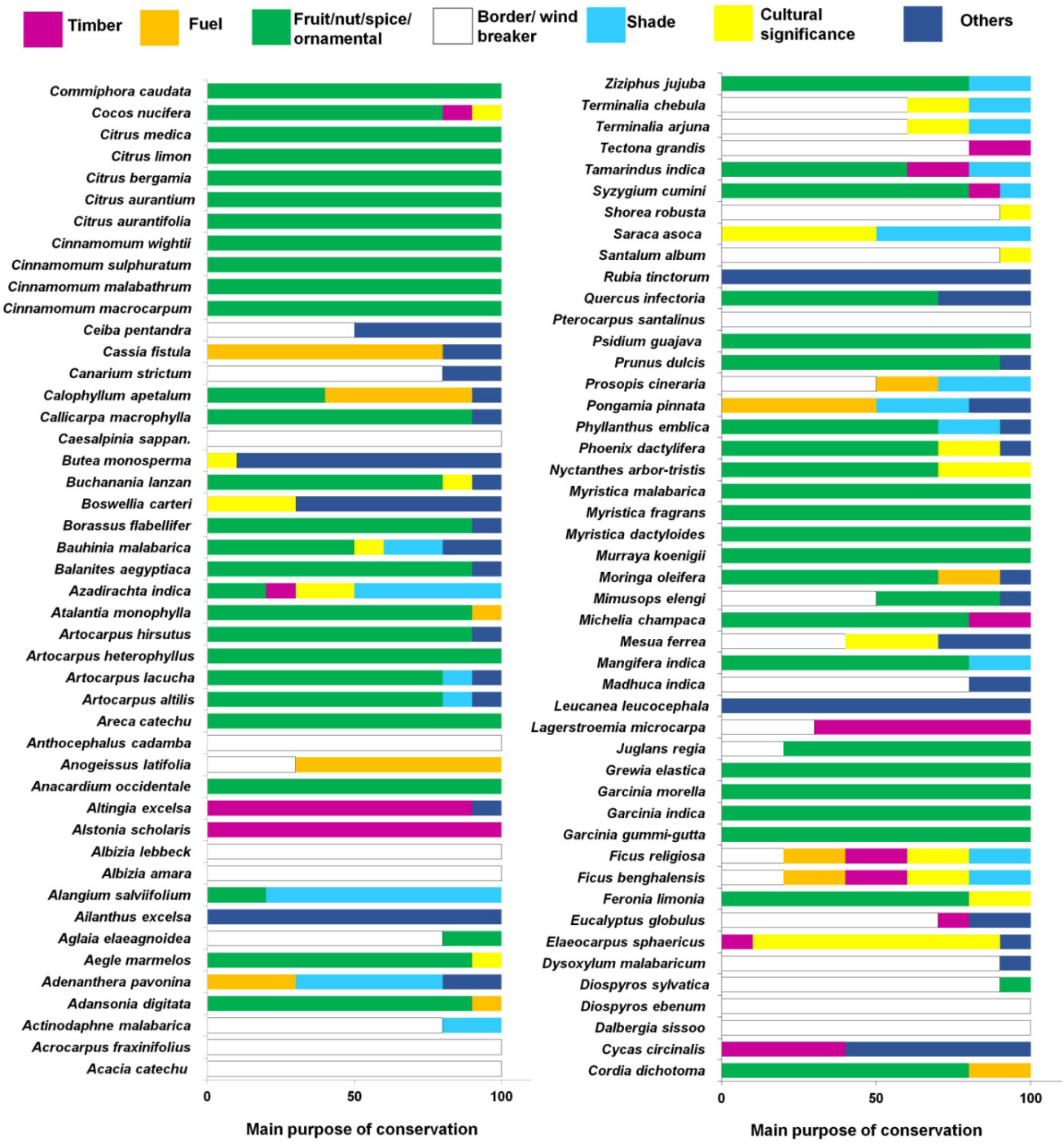


Figure 3: Use categories of tree species conserved *circa situm* by the farmers of Central Western Ghats

Acrocarpus fraxinifolius, *Actinodaphne malabarica*, *Aglaia elaeagnoidea*, *Albizia amara*, *A. lebbeck*, *Anthocephalus cadamba*, *Caesalpinia sappan*, *Dalbergia sissoo*, *Diospyros ebenum*, *D. sylvatica*, *Dysoxylum malabaricum*, *Madhuca indica*, *Pterocarpus santalinus*, *Santalum album*, *Shorea robusta* and *Tectona grandis* (80–100%). *Alstonia scholaris*, *Altingia excelsa*, *Lagerstroemia microcarpa* are majorly grown as a border crops for the demarcation of land holdings. *Elaeocarpus sphaericus* is being conserved for its biocultural significance of flowers (however exotic species such as *A. digitata*, *A. occidentale*, *My. fragrans*, *P. guajava*, *Q. infectoria* species were preferred for conservation suggesting a change in the species composition of the agro ecosystesms) The opinion of farmers is also consistent with the report of Aerts *et al.*, (2011) and Maheswarappa *et al.*, (2021). The first report on *circa situm* conservation of tree species in India specifically in the Karnataka coffee agroforestry system was reported by Maheswarappa *et al.*, (2021) and they observed

that many species are conserved for timber production, shade for coffee plantations, pollination, enhancement of quality of coffee beans, pest control were cited by farmers for retaining the native species.

Conclusion

Tree species are one of the important components of terrestrial ecosystems and agrobiodiversity. Karnataka state is endowed with a diverse climate, topography and soils and is reported to have the highest number of tree species. The present study has confirmed the conservation of vast diversity of tree genetic resources *circa situm*. The farmers conserved trees for their tangible and intangible benefits and termed "conservation through use", many species recorded in the present study area are being conserved due to their use as timber, fruit/nut/spice/ornamental, border/windbreaker, fuel, cultural significance, shade and other (gum, resin, soil conservation, etc.). This study emphasizes that there is a necessity from the government sector to encourage farmers practicing *circa situ* tree conservation through due recognition and remuneration for sustainability of the conservation practice.

Reference

Aerts, R., Hundera, K., Berecha, G., Gijbels, P., Baeten, M., Van Mechelen, M., Hermy, M., Muys, B., & Honnay, O. (2011). Semi-forest coffee cultivation and the conservation of Ethiopian Afromontane rainforest fragments. *For. Ecol. Manag.*, **261(6)**: 1034-1041.

Agrawal, A., Gowthami, R., Srivastava V., & Chander, S., (2023). *Trans situ* conservation of PGR. In: RK Gautam *et al*., (Eds.) *Plant Genetic Resources Management: Theory and Practices*. The Director, ICAR-NBPGR, New Delhi, India, pp 44-52.

Arnold, J. M., & Dewees, P. A. (1995). Tree management in farmer strategies: responses to agricultural intensification. Oxford (UK), Oxford University Press, 1995.

Boshier, D. H., Gordon, J. E., & Barrance, A. J. (2004). Prospects for circa situm tree conservation in Mesoamerican dry forest agro-ecosystems. In: Biodiversity conservation in Costa Rica. University of California Press, Berkeley, USA, 210-226.

Brush, S. B. (1991). A farmer-based approach to conserving crop germplasm. *Econ. Bot.*, **45(2)**: 153-165.

Cazzolla Gatti, R., Reich, P.B., Gamarra, J.G., Crowther, T., Hui, C., Morera, A., Bastin, J.F., De-Miguel, S., Nabuurs, G.J., Svenning, J.C. and Serra-Diaz, J.M. (2022). The number of tree species on Earth. *Proceedings of the National Academy of Sciences*, **119(6)**: e2115329119.

Cristóbal-Pérez, E. J., Fuchs, E. J., Lobo, J., & Quesada, M. (2022). Genetic diversity, asexual reproduction and conservation of the edible fruit tree *Spondias purpurea* L. (Anacardiaceae) in the Costa Rican tropical dry forest. *Plos one*, **17(11)**: e0277439.

Dawson, I.K., Guariguata, M.R., Loo, J., Weber, J.C., Lengkeek, A., Bush, D., Cornelius, J., Guarino, L., Kindt, R., Orwa, C. and Russell, J., (2013). What is the relevance of smallholders' agroforestry systems for conserving tropical tree species and genetic diversity in *circa situm*, *in situ* and *ex situ* settings? A review. *Biodiversity Conserv.*, **22**: 301-324.

Dhanya, B., Viswanath, S., & Purushothaman, S. (2013). Crop yield reduction in ficus agroforestry systems of Karnataka, Southern India: perceptions and Realities. *Agroecol. Sustain. Food Syst.*, **37(6)**: 727-735.

Dhyani, A., Bindu, S., Abinlal, K. C., & Anilkumar, C. (2023). Reintroduction of Buchanania barberi, a critically endangered tree endemic to the south Western Ghats of Kerala, India. *Plants, People, Planet*, **5(4)**: 502-507.

FAO (2014). The state of the world's forest genetic resources. Rome, Italy.

FSI (2019). India State of Forest Report, Volume II, Forest Survey of India, Dehradun.

Gowthami, R., Sharma, N., Pandey, R., & Agrawal, A. (2021). Status and consolidated list of threatened medicinal plants of India. *Genet. Resour. Crop Evol.*, **68(6)**: 2235-2263.

Han, L., Love, K., Peace, B., Broadhurst, L., England, N., Li, L., & Bush, D. (2020). Origin of planted Eucalyptus benthamii trees in Camden NSW: checking the effectiveness of *circa situm* conservation measures using molecular markers. *Biodiversity Conserv.*, **29(4)**: 1301-1322.

Herrera-Cabrera, B. E., Campos Contreras, J. E., Macías-Cuéllar, H., Delgado-Alvarado, A., & Salazar-Rojas, V. M. (2020). Beyond the traditional home garden: a *circa situm* conservation experience of *Laelia anceps* subsp. *dawsonii* f. *chilapensis* Soto-Arenas (Orchidaceae). *Environment, Development and Sustainability*, **22**: 1913-1927.

IUCN (2023). The IUCN red list of threatened species. Version 2020-1. *https://www.iucnredlist.org. Accessed on 4.04.2020.*

Maheswarappa, V., Vasudeva, R., & Hegde, R. (2022). Circa situm conservation of coffee agroforests: farmer's perception in Kodagu landscape of Karnataka, India. *Trop. Ecol.*, **63(1)**: 104-112.

Oli, B. N., Treue, T., & Larsen, H. O. (2015). Socio-economic determinants of growing trees on farms in the middle hills of Nepal. *Agrofor. Syst.*, **89**: 765-777.

Sasidharan, N. (2003). Red listed threatened tree species in Kerala: a review. In *Conservation and Research Needs of the Rare, Endangered and Threatened (RET) Tree Species in Kerala Part of the Western Ghats. Proc. Workshop. KFRI, Peechi* (pp. 1-12).

Seth, M. K. (2003). Trees and their economic importance. *Bot. Rev.*, **69(4)**: 321-376.

Van Zonneveld, M., Thomas, E., Castañeda-Álvarez, N. P., Van Damme, V., Alcazar, C., Loo, J., & Scheldeman, X. (2018). Tree genetic resources at risk in South America: A spatial threat assessment to prioritize populations for conservation. *Divers. Distrib.*, **24(6)**: 718-729.

Vasudeva, R. (2022). A Novel Circa situm Approach to Conserve Forest Genetic Resources of the Western Ghats. *Indian J. Plant Genet. Resources*, **35(03)**: 37-40.

Vasudeva, R., Sthapit, B., Salma, I., Changtragoon, S., Arsanti, I.W., Gerten, D., Dum-ampai, N., Rajan, S., Dinesh, M.R., Singh, I.P. and Singh, S.K. & Rao, V. R. (2015). Use values and cultural importance of major tropical fruit trees: An analysis from 24 village sites across South and South-East Asia. *Indian J. Plant Genet. Resources*, **28(1)**: 17-30.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.11

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

A Note on Taxonomy and Genetic Resource Potential of *Oryza meyeriana* var. *indandamanica*, a Rare Wild Relative of Rice from the Andaman Islands

K Pradheep[1*], R Parimalan[2], K Joseph John[1], VA Muhammed Nissar[3], I Jaisankar[4], PP Thirumalaisamy[1] and M Latha[1]

Abstract

The narrow endemic taxon, *Oryza meyeriana* (Zoll. & Moritzi) Baill. var. *indandamanica* (J.L.Ellis) Veldkamp, was often merged with either *O. meyeriana* (Zoll. & Moritzi) Baill. or *O. granulata* Nees & Arn. ex Watt. or all three taxa were subsumed to *O. meyeriana*. A critical analysis of protologues, herbarium specimens and earlier taxonomic works pertaining to these three taxa coupled with the detailed morphological study of germplasm (IC641181) of var. *indandamanica*, which was originally collected from the Saddle Peak National Park of the North Andaman Island, was undertaken. Morphological studies indicated that var. *indandamanica* fell well within the circumscription of var. *meyeriana*. However, chloroplast genome sequence data reveals its closeness towards var. *granulata*, compared with var. *meyeriana*. Our views agree to the merger of all these three taxa into one are presented. Also, the genetic resource potential of the studied accession is discussed.

Keywords: Andaman wild rice, Chloroplast genome sequence, *Oryza meyeriana* complex, Narrow endemic taxon, Saddle Peak National Park.

[1]ICAR-National Bureau of Plant Genetic Resources, Regional Station, Thrissur, Kerala, India.

[2]ICAR-National Bureau of Plant Genetic Resources, New Delhi, India.

[3]ICAR-Indian Institute of Spices Research, Kozhikode, Kerala, India.

[4]ICAR-Central Island Agricultural Research Institute, Port Blair, Andaman & Nicobar Islands, India.

***Author for correspondence:**

K.Pradheep@icar.gov.in

Received: 11/05/2023 **Revised:** 23/10/2023

Accepted: 26/10/2023

How to cite this article: Pradheep, K., Parimalan, R., John, K.J., Nissar, V.A.M., Jaisankar, I., Thirumalaisamy, P.P., Latha, M. (2023). A Note on Taxonomy and Genetic Resource Potential of *Oryza meyeriana* var. *indandamanica*, a Rare Wild Relative of Rice from the Andaman Islands. Indian J. Plant Genetic Resources. 36(3), 422-428.**DOI:** 10.61949/0976-1926.2023.v36i03.11

Introduction

A trekking for crop wild relatives' survey and their germplasm collection was carried out at the Saddle Peak National Park (SPNP) of North Andaman Island in January 2020. In the underneath of the evergreen forest canopy at an altitudinal range of 330 to 590 m above MSL for a stretch of around two kilometers (13.1637–13.1780° N, 93.0063–93.0125° E), the authors came across a dwarf but stiff bambusoid tuft-forming grass with erect culms and apparently unbranched spicate inflorescence with small, awnless spikelets. This population with over 100 clumps/plants was spotted in highly shady steep-sloped stony terrain, having well-drained loamy soils with rich litter. It was identified as wild rice, *Oryza meyeriana* (Zoll. & Moritzi) Baill. var. *indandamanica* (J.L.Ellis) Veldkamp, based on the earlier floristic records of this park. Known in just two localities, Rutland (type locality) and SPNP, the germplasm of this narrow endemic taxon was collected for the first time from the latter area. The collected germplasm (IC641181) is maintained in the Field Genebank (FGB) at Thrissur, and seeds were also conserved in the National Genebank at NBPGR, New Delhi, for long-term storage.

O. indandamanica J.L.Ellis was often considered an ecotype/variant/synonym of *O. granulata* Nees & Arn. ex Watt (e.g., Rao, 1989; Vaughan, 1989, 1994; Khush *et al*., 1990) or identical with var. *meyeriana* (Veldkamp, 1988) or often all 3 infraspecific taxa names subsumed/merged under *O. meyeriana* (Zoll. & Moritzi) Baill. (Clayton *et al*., 2006; POWO, 2023) or *O. granulata* (Gong *et al*., 2000;

Lu *et al*., 2001). Nevertheless, using the total genomic DNA hybridization approach, Aggarwal *et al*. (1997) concluded that the genomes of these three species in *meyeriana* complex were highly divergent and distinguishable. Biogeographically, *Oryza granulata* is distributed in South Asia, SE Asia and SW China, while *O. meyeriana* is confined to SE Asia only. These species together with the Neo Caledonian endemic species, *O. neocaledonica* Morat, form the *Oryza meyeriana* complex (2n=24) designated with the GG genome group.

JL Ellis, the original author of *O. indandamanica* had distinguished the species only with *O. granulata* (and not with *O. meyeriana*), that too on feeble, and sometimes on arbitrary characters (e.g., almost unbranched panicle, absence of fertile lemma awn) (Ellis, 1985). It appears that he relied heavily on expert opinion (vide Ellis, 1994) rather than studying the protologue of allied species and associated herbarium specimens and literature. JF Veldkamp reduced this taxon to a varietal status under *O. meyeriana*, citing that the differences observed were insufficient to maintain as a distinct species (Veldkamp, 1991). Establishing the correct identity of the collected germplasm is crucial as misidentification of species and/or instability or non-universality in the application of taxon names greatly limits the value of plant genetic resources.

With the advent of next-generation sequencing technologies, chloroplast genome sequences had proven to be effective molecular resources for aspects related to species identification and phylogenetic studies (Dong *et al*., 2012), owing to their uniparental inheritance (occasionally paternal as well), lacking recombination, and lower rates of adaptive evolution than that of nuclear genomes (Neale and Sederoff, 1989; Rogalski *et al*., 2015; Daniell *et al*., 2016; Chung et al., 2023; Heinke, 2023). The highly variable regions in the chloroplast genomes can serve as DNA markers for the accurate and reliable identification of plant species. Therefore, both the morphological studies and molecular tools using chloroplast genome sequences were employed to arrive at a reasonable conclusion about the taxonomy of *Meyeriana* complex, in general, and the identity of the collected germplasm, in particular.

Material and Methods

A detailed morphological study of field-grown germplasm accession IC641181 was made and compared with the botanical description given in the protologue of *O.*

Table 1: Comparison of key morphological characters of studied accession with three taxa (Ellis 1985; Duistermaat 1987; Chang 1988; Veldkamp 1991; Vaughan 1994)

#	*Characters*	*IC641181*	*var. indandamanica*	*var. meyeriana*	*var. granulata*
1.	Fruiting spikelet	Oblong; 6.93 mm long; Length Width ratio 4.61	-----	Oblong-ovate lanceolate; more than (6.00–) 6.4 (–7) mm long; LW ratio 3.10–6.40	Elliptic; less than (6.00–6.4) (–7) mm long; LW ratio 2.10–2.70
2.	Caryopsis length (mm)	5.21	5.00	4.10–7.30	3.40–4.10
3.	Blade width (cm)	1.26	0.80	0.80–3.20	0.70–2.00
4.	Ligule	Collar-shaped, erose; c. 1.50 mm	1.00 mm; erose	Collar-shaped, erose; 1.00–5.00 mm	Collar-shaped, erose; 0.50–2.00 mm
5.	Auricle size (mm)	0.90 × 0.20	-----	1.00 × 0.20	0.50–3.00 × 0.20
6.	Sterile lemma length (mm)	1.76	1.50–2.50	0.15–2.45	0.05–1.40
7.	Sterile lemma shape	Deltoid - triangular	Linear-acuminate	Deltoid - triangular	Deltoid - triangular

Table 2: Evolutionary divergence estimates (distance matrix) for the multiple sequence alignment performed using the whole chloroplast genome among the six taxa of *Oryza* spp.

	1	2	3	4	5	6	7
1	0.010219						
2	0.000832	0.010621					
3	0.010904	0.014880	0.011181				
4	0.000228	0.010225	0.000868	0.010941			
5	0.000221	0.010292	0.000898	0.010956	0.000302		
6	0.010219	0.000000	0.010621	0.014880	0.010225	0.010292	
7	0.011035	0.015011	0.011312	0.000252	0.011072	0.011087	0.015011

1: *O. meyeriana* var. *indandamanica* (135,917 bp, Acc. No. OX485189, developed through this study); 2: *O. brachyantha* (134,644 bp, MT726939); 3: *O. neocaledonica* (135,950 bp, NC_053276 / MT726926); 4: *O. australiensis* (135,193 bp, MT726929); 5: *Oryza meyeriana* (136,133 bp, NC_034765 / KF359921); 6: *O. granulata* (135,942 bp, KF359920); 7: *O. brachyantha* (134,644 bp, KF359917); 8: *O. australiensis* (135,222 bp, KF359916). Here, *O. australiensis* and *O. brachyantha* were used as out groups.

indandamanica. Observation and data collection was done on ten plants by taking the average of three measurements for each plant for the characters of taxonomic significance (Table 1) to the genus *Oryza*. Simultaneously, critical analysis of protologues, the herbarium specimens of var. *granulata*, var. *meyeriana* and var. *indandamanica,* including type(s) housed at Kew (K), Leiden (L), Kolkata (CAL) and Port Blair (PBL) and taxonomic revisionary works (Duistermaat 1987; Chang 1988; Veldkamp 1991; Vaughan 1994) was made.

Chloroplast Assembly and Multiple Sequence Alignment

Freshly harvested leaves from the field-grown germplasm accession IC641181 were used for DNA extraction. In brief, approximately 1.5 g of the leaf samples were taken in a porcelain mortar and ground to a fine powder using liquid nitrogen. These powdered tissues were homogenized using 15 mL of 2% CTAB extraction buffer and the high-quality DNA was extracted using the protocol as reported earlier (Stewart and Via, 1993). The quality and quantity of the DNA was estimated using a 0.8% agarose gel electrophoresis and a Qubit 4.0 fluorometer. Quantified DNA was utilized thereafter for library preparation and sequencing using Novaseq6000 platform of the Illumina to obtain 2x150 bp paired-end data. The raw data obtained was subjected to adapter removal using TrimGalore v0.6.7 (Martin, 2011), and the quality check was done using FastQC v0.11.9 (Andrews, 2010) for the generated data. This short read data was used to generate the chloroplast assembly for the reported taxon using GetOrganelle v1.7.4.45 (Jin *et al*., 2020). Chloroplast genome annotation was performed using the GeSeq tool (Tillich *et al*., 2017); annotated information was used to derive the organelle genome map using OGDRAW v1.3.1 (Greiner *et al*., 2019). The generated whole chloroplast genome was aligned using ClustalW of the MEGA v11.0 with default parameters (Tamura *et al*., 2021), along with whole chloroplast genome sequence of other taxa of *Meyeriana* complex (downloaded from public domain) *viz*., *Oryza meyeriana* (136,133 bp; NC_034765.1), *O. granulata* (135,942 bp; KF359920.1), and *O. neocaledonica* (135,950 bp; NC_053276.1). Additionally, the whole chloroplast genome

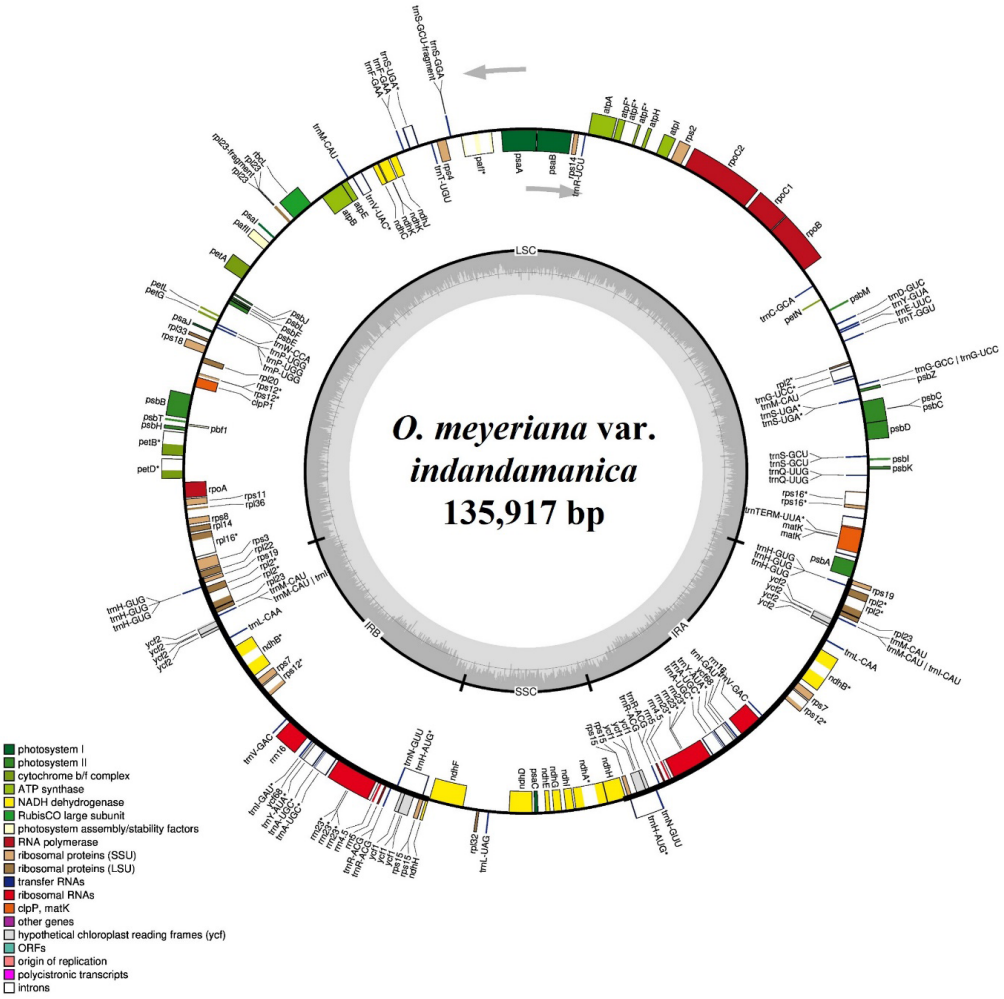


Figure 1: Single contig circular assembly of the chloroplast genome of 135,917 bp length for *Oryza meyeriana* var. *indandamanica* (IC641181). For sequence information please refer to the public database with accession number OX485189.1.

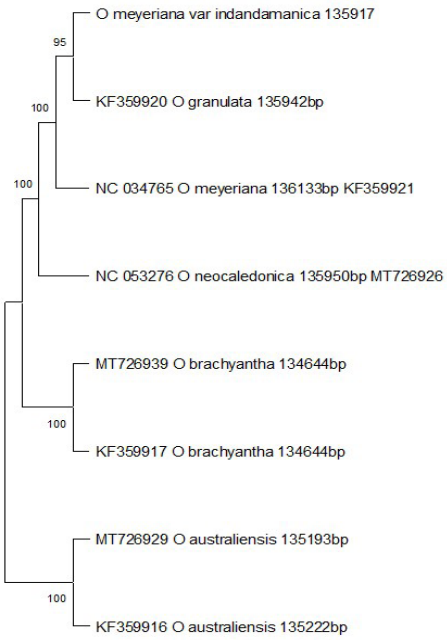


Figure 2: Cladogram derived using multiple sequence alignment for the whole chloroplast genome sequence for the represented taxa. Values at nodes indicate bootstrap values in percentage. For *O. meyeriana* var. *indandamanica*, the chloroplast genome sequence has been generated through the present study.

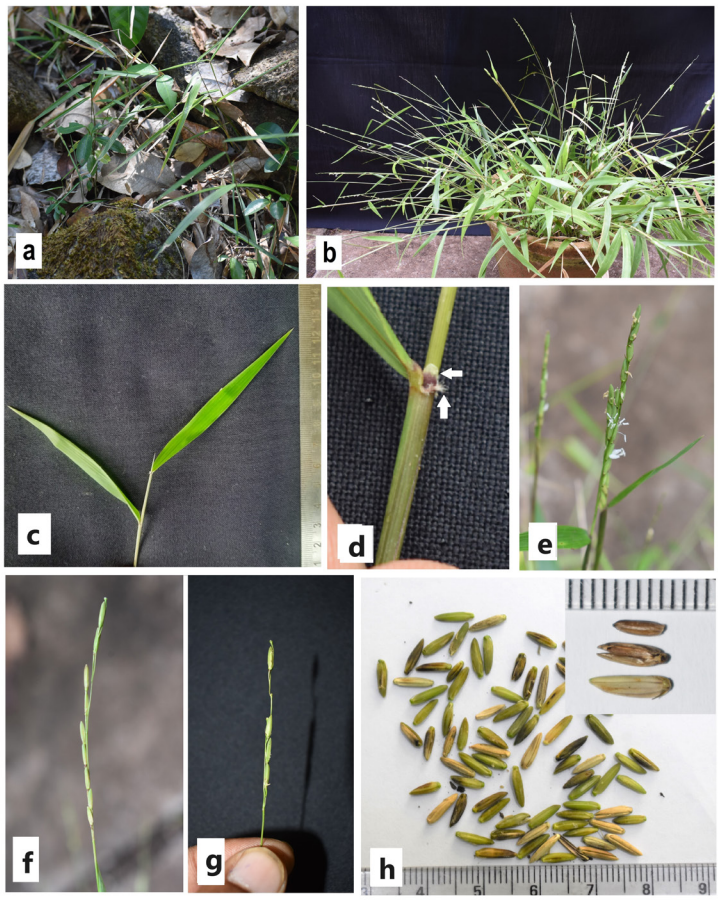


Plate 1: *Oryza meyeriana* var. *indandamanica* from SPNP. a. Plants in natural habitat; b. *Ex-situ* regeneration at Thrissur; c. Leaves - lower surface (left one) and upper surface (right one); d. Ligule and auricle of leaf; e. Inflorescence at anthesis; f. Inflorescence at dough stage; g. Inflorescence at spikelet maturity; h. Physiologically mature (pale green) and mature dry seeds (inset: seed, hull and kernel).

sequence of *O. australiensis* (135,222; KF359916.1 and 135,193 bp; MT726929.1, EE genome of *Officinalis* complex) and *O. brachyantha* (134,644 bp; MT726939.1 and KF359917.1, FF genome of *Officinalis* complex) were used as outgroup for the *Meyeriana* complex in this study. The evolutionary divergence estimates generated in the software was used to derive a cladogram using the maximum likelihood method and general time reversible model (Nei and Kumar, 2000). The confidence for the generated tree was assessed through the bootstrap option with 2000 replicates (Felsenstein, 1985).

Results and Discussion

A comparison of key morphological characters of studied accession with three taxa (Table 1) indicated that the SPNP population of var. *indandamanica* falls well under the circumscription of var. *meyeriana*, instead of var. *granulata*. The observed key characters, *viz*., fruiting spikelets oblong, 4.61 times as long as wide, and caryopsis 5.21 mm long, readily differentiate from var. *granulata* (having an elliptic fruiting spikelet which is only 2.10–2.70 times as long as wide, and caryopsis 3.40–4.10 mm long) (vide Duistermaat, 1987) (Plate 1). The characters given in the protologue of var. *inandamanica* (Ellis, 1985) also fit well within the range of characters recognized for var. *meyeriana*, except for the shape of the sterile lemma which is the case for the usage of inappropriate terminology. However, the character 'ribs in lower internode' as mentioned for the *meyeriana* complex (Duistermaat, 1987) was not evident in both the Andaman populations. Also, spikelets of Andaman materials are nearly smooth and hardly granulate, unlike that of the other two species (vide Vaughan, 1994) while Duistermaat (1987) didn't give weightage for this micromorphological trait. A few taxonomic characteristics of the SPNP population such as length of inflorescence (excl. peduncle) (7–9 *vs*. 5 cm in var. *inandamanica*) and floret (6.93 *vs*. 5 mm) and tufted habit (in natural habitat) do not match with the protologue information indicating the role of environmental factors modifying the phenotypic expression of plants. Herbarium studies also revealed that the characteristics such as habit, leaf shape and size, panicle length and branching nature used to study *meyeriana* complex had limited taxonomic value as the environment often modifies their expression.

The single-contig circular chloroplast genome of size 135,917 bp generated is given in Figure 1. Functional annotation using GeSeq tool revealed the presence of 125 genes comprising four rRNA genes, 34 tRNA genes and 87 mRNA genes. The list of unique genes among the annotated genes exhibited a conserved nature when compared with the other *Oryza* chloroplast genomes reported by Gao *et al*. (2019). Although our annotation had revealed the presence of *ycf1* and *ycf2* (Supplementary material 1), the absence of the genes *ycf1*, ycf2, and *ycf15* in the *Oryza* chloroplast genomes as documented by Gao *et al*. (2019) underscores the need for functional validation of *ycf1* and *ycf2*. Positively selected genes for shade tolerance in *Oryza* genus *viz*., *ndhD*, *rbcL*, *ndhH*, *psbD*, and *psbH* (Gao *et al*., 2019), were also identified in this taxon which is a shade tolerant one. The whole chloroplast genome sequence alignment as represented in the cladogram (Figure 2) indicated the closeness of var. *indandamanica* with var. *granulata* than var. *meyeriana* among the *Meyeriana* group. Also, the evolutionary divergence estimates (distance matrix) used to derive this cladogram suggests that the three taxonomical varieties of *O. meyeriana*, *viz*., var. *meyeriana*, var. *granulata*, and var. *indandamanica* were not sufficiently divergent from an evolutionary perspective (Table 2). This is in corroboration with the earlier report (Kumagai *et al*., 2010) on the phylogenetic study of the genus *Oryza* using chloroplast DNA sequences.

Foregoing studies indicated that the studied accession matches with var. *meyeriana* morphologically, but clusters with var. g*ranulata* at molecular level, indicating that a separate taxonomic identity as var. *indandamanica* is untenable. This further stems from the key taxonomic character 'spikelet size and shape', which showed continuous variation and inconsistency (Gong *et al*. 2000; Vaughan 2005). Different workers have set varied spikelet length values for distinguishing *O. meyeriana* from *O. granulata*, *viz*., > 6.4 mm (Vaughan, 1994), > 6.8 mm (Duistermaat, 1987) and >7 mm (Chang, 1988) indicating the lack of consensus owing to continuous variation exhibited for this trait. Wide hybridization experiments (Gong *et al*., 2000) between *granulata* and *meyeriana* resulted in high crossability (34–39% seed set) with relatively high fertility of interspecific hybrids, which denotes a very high genomic affinity and very limited reproductive isolation. Earlier, Khush *et al*. (1990) undertook morphological and cytological studies of the F_1 hybrids between *O. granulata* and *O. indandamanica* and concluded that both had identical genomes, and the latter could be regarded as an ecotype of *O. granulata* only. Based upon the principle of priority of ICN, the name *O. meyeriana* has precedence over *O. granulata*, therefore, become the currently accepted name.

With reference to the genetic resource potential of the studied accession, the habitat ecology of the studied population indicates the potential for tolerance to shade (as thriving in almost full shade) and drought (as occurring on steep stony slopes, blades roll inwards in response to stress). Mandal *et al*. (1989) reported the presence of wax-like coating over the culm and densely fibrous root system with abundant xylem strands in *O. indandamanica* contributing to drought tolerance. It takes only 16 days from anthesis to seed maturity (while rice takes about 30 days), and afterwards, the color of the lemma and palea changes from pale green to straw yellow within a day and

sheds immediately. For this reason, it is very difficult to find mature grains on culms and therefore needs white clothing underneath to collect seeds. At Thrissur, the plant produced abundant tillers (up to 40), and flowers year-round, and they open at 10 AM throwing out far protruding white stigmas. The *meyeriana* complex is known for higher seed protein, including glutelin, and is a known source of bacterial leaf blight resistance. The studied accession has potential for studies involving biotic stress tolerance traits, for which only limited variability in rice is available (e.g., false smut, neck blast and stem borer). Nevertheless, it was found infected with brown spot (*Biploris oryzae*) under Thrissur conditions. Although *meyeriana* complex forms only a tertiary genepool of rice, advances in molecular biological techniques hold promise to broaden the crop genepool. Understanding the physiological, genetic and molecular basis of stress tolerance and its application in rice improvement programme is urgently required.

Though being a major food crop genus, general disagreement between rice researchers and taxonomists regarding the issues pertaining to synonymy in a few species of *Oryza* needs an urgent relook. A few Indian examples were *O. nivara vs O. rufipogon*; *O. minuta vs O. officinalis*; *O. malampuzhaensis vs O. officinalis*; and ambiguous taxa such as *O. perennis* and *O. spontanea*. The integrative taxonomy approach using information from various sources such as ecological data, molecular data, and detailed morphological characters would be helpful in solving species limits.

Sequence Availability

The assembled chloroplast genome sequence for *Oryza meyeriana* var. *indandamanica* (IC641181) of 135,917 bp is available through the public database on the accession number OX485189.1 (PRJEB61731 or INRP000060).

Acknowledgements

The authors are thankful to the Director, ICAR-NBPGR, New Delhi and the Head, Division of Plant Exploration and Germplasm Collection, ICAR-NBPGR New Delhi for the institutional support through which the exploration was conducted. Thanks to all the Department of Environment & Forest, Andaman & Nicobar Administration officials, who were very supportive in collecting this germplasm from SPNP. Authors K Pradheep and Parimalan R are thankful to the Indian Council of Agricultural Research for the 'Incentivizing research in agriculture' scheme for the molecular part of this study was performed through this research grant support.

References

Aggarwal RK, DS Brar and GS Khush (1997) Two new genomes in the *Oryza complex* identified on the basis of molecular divergence analysis using total genomic DNA hybridization. *Mol. Gen. Genet.* **254**: 1-12.

Andrews S (2010) FastQC: a quality control tool for high throughput sequence data. Available online at *http://www.bioinformatics.babraham.ac.uk/projects/fastqc (last accessed on 24.04.2023).*

Chang TT (1988) Taxonomic key for identifying the 22 species in the genus *Oryza*. International Rice Research Institute, Los Banos, Philippines. *IRRI Newsl.* **13**: 4-5.

Chung KP, EG Duran, S Ruf, P Endries and R Bock (2023) Control of plastid inheritance by environmental and genetic factors. *Nat. Plants* **9**: 68-80.

Clayton WD, KT Harman and H Williamson (2006) *World Grass Species - Synonymy Database* The Board of Trustees of the Royal Botanic Gardens, Kew. Available online: *http://www.kew.org/data/grasses-db.html (last accessed on 15.04.2023)*

Daniell H, CS Lin, M Yu and WJ Chang (2016) Chloroplast genomes: diversity, evolution, and applications in genetic engineering. *Genome Biol.* **17**: 134. *doi: 10.1186/s13059-016-1004-2.*

Dong W, J Liu, J Yu, L Wang, S Zhou (2012) Highly variable chloroplast markers for evaluating plant phylogeny at low taxonomic levels and for DNA barcoding. *Plos One* **7**: e35071. *doi: 10.1371/journal.pone.0035071.*

Duistermaat L (1987) A revision of *Oryza* (Gramineae) in Malesia and Australia. *Blumea* **32(1)**: 157-193.

Ellis JL (1985) *Oryza indandamanica* Ellis, a new rice plant from islands of Andamans. *Bull. Bot. Surv. India* **27(1-4)**: 225-227.

Ellis JL (1994) *Oryza indandamanica* Ellis – The wild Andaman rice. *J. Econ. Taxon. Bot.* **18(1)**: 245-246.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evol.* **39**: 783-791.

Gao LZ, YL Liu, D Zhang, W Li, J Gao, Y Liu, K Li, C Shi, Y Zhao, YJ Zhao, JY Jiao, SY Mao, CW Gao and EE Eichler (2019) Evolution of *Oryza* chloroplast genomes promoted adaptation to diverse ecological habitats. *Commun. Biol.* **2**: 278. *https://doi.org/10.1038/s42003-019-0531-2*

Gong Y, T Borromeo and BR Lu (2000) A biosystematic study of the *Oryza meyeriana* complex (Poaceae). *Plant Syst. Evol.* **224**: 139-151.

Greiner S, P Lehwark and R Bock (2019) OrganellarGenomeDraw (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. *Nucleic Acids Res.* **47**: W59-W64.

Heinke L (2023) Chilling paternal chloroplasts. *Nat. Rev. Mol. Cell Biol.* **24:** 166.

Jin JJ, WB Yu, JB Yang, Y Song, CW dePamphilis, TS Yi and DZ Li (2020) GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biol.* 21: 241. *https://doi.org/10.1186/s13059-020-02154-5*

Khush GS, DS Multani, GV Vergara and DS Brar (1990) Taxonomic status of *Oryza indandamanica*. *Rice Genet. Newsl.* **7**: 141-142.

Kumagai M, L Wang and S Ueda (2010) Genetic diversity and evolutionary relationships in genus *Oryza* revealed by using highly variable regions of chloroplast DNA. *Gene* **462(1-2)**: 44-51.

Lu BR, GE Song, SANG Tao, CHEN Jia-Kuan and HONG De-Yuan (2001) The current taxonomy and perplexity of the genus *Oryza* (Poaceae). *J. Syst. Evol.* **39(4)**: 373-388.

Mandal AB, T Ram, ND Majumdar and TVRS Sharma (1989) *Oryza indandamanica* sp. nov. -Morphoanatomical perspective. *Phytobreedon* **5(1)**: 25-32.

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J* **17**: 10-12.

Neale DB and RR Sederoff (1989) Paternal inheritance of chloroplast

DNA and maternal inheritance of mitochondrial DNA in loblolly pine. *Theor. Appl. Genet.*, **77**: 212-216.

Nei M and S Kumar (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, New York, 348 p.

POWO (2023) *Plants of the World Online*. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; *http://www.plantsoftheworldonline.org/ Retrieved 21.04.2023*.

Rao MKV (1989) A critical note on the Andaman wild rice. *J. Econ. Taxon. Bot.* **13(2)**: 249-253.

Rogalski M, L do Nascimento Vieira, HP Fraga and MP Guerra (2015) Plastid genomics in horticultural species: importance and applications for plant population genetics, evolution, and biotechnology. *Front Plant Sci*. **6**: 586. *doi: 10.3389/fpls.2015.00586*.

Stewart Jr CN and LE Via (1993) A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. *Biotechniques* **14(5)**: 748-750.

Tamura K, G Stecher and S Kumar (2021) MEGA 11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol*. **38**: 3022-3027.

Tillich M, P Lehwark, T Pellizzer, ES Ulbricht-Jones, A Fischer, R Bock and S Greiner (2017) GeSeq – versatile and accurate annotation of organelle genomes. *Nucleic Acids Res*. **45**: W6-W11.

Vaughan DA (1994) *The Wild Relatives of Rice*: *A Genetic Resources Handbook*. International Rice Research Institute, Manilla, 137 p.

Vaughan DA, KI Kadowaki, A Kaga and N Tomooka (2005) On the phylogeny and biogeography of the genus *Oryza*. *Breed. Sci.* **55(2)**: 113-122.

Vaughan DA (1989) The genus *Oryza* L.: current status of taxonomy. *IRRI Res. Paper Ser.* 138, International Rice Research Institute, Manilla, 21 p.

Veldkamp JF (1988) XI. New Records. *Flora Malesiana Bull.* **10**: 27-31.

Veldkamp JF (1991) Miscellaneous notes on Southeast Asian Gramineae. VI. *Blumea* **36(1)**: 179-181.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.12

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Effects of Different Treatments on Seed Germination and Breaking Seed Dormancy in Wild *Luffa* Species

Arvind Nagar[1], Amish K. Sureja[1*], Chithra Pandey[2], Manjusha Verma[3], Lalit Arya[3], Nakul Gupta[4] and A.D. Munshi[1]

Abstract

The wild species of *Luffa* require prolonged duration to germinate under normal environmental conditions due to prevalence of seed dormancy owing to variation in their seed size, degree of seed hardiness and variable lignin content in seed coat. Therefore, present study was conducted to identify best pre-sowing treatment for seed germination, seedling growth in two wild *Luffa* species namely *Luffa graveolens* Roxb and *L. echinata* Roxb. Seeds of four accessions of both the species (three of *L. graveolens* and one of *L. echinata*) were exposed to scarification, chemical treatment with gibberellic acid (GA_3) and potassium nitrate (KNO_3) at different concentrations. Among various applied treatments, scarification + GA_3 @ 200 ppm was found most effective in *L. graveolens* and scarification + KNO_3 @ 0.2% for *L. echinata* for breaking of seed dormancy in freshly harvested seed. The seedling vigor in terms of shoot length, root length, seedling dry weight were recorded maximum in scarification + GA_3 @ 200 ppm for *L. graveolens* and scarification + KNO_3 @ 0.2% for *L. echinata* for both fresh as well as one year old seed of same lot. It reflects the presence of hard seed coat and physiological dormancy in wild *Luffa* species, which can be broken to a greater extent with an additional pre-sowing treatment, to enhance seed germination and vigor for uniform plant stand.

Keywords: *Luffa* wild species, Dormancy, Seed priming, Scarification, Gibberellic acid, Potassium nitrate.

[1]Division of Vegetable Science, ICAR-IARI, Pusa, New Delhi, Delhi, India

[2]Division of Germplasm Conservation, ICAR-NBPGR, Pusa, New Delhi, Delhi, India

[3]Division of Genomic Resources, ICAR-NBPGR, Pusa, New Delhi, Delhi, India

[4]ICAR-IIVR, Varanasi, Uttar Pradesh, India

***Author for correspondence:**

aksureja@gmail.com

Received: 12/06/2023 **Revised:** 12/07/2023

Accepted: 17/07/2023

How to cite this article: Nagar, A., Sureja, A.K., Pandey, C., Verma, M., Arya, L., Gupta, N. and Munshi, A.D. (2023). Effects of Different Treatments on Seed Germination and Breaking Seed Dormancy in Wild *Luffa* Species. Indian J. Plant Genetic Resources. 36(3), 429-433.

DOI: 10.61949/0976-1926.2023.v36i03.12

Introduction

Genus *Luffa* belongs to family Cucurbitaceae, which includes more than eight species, among them *Luffa aegyptica* Mill., *L. acutangula* (Roxb.) L., *L. hermaphrodita, L. echinata* Roxb., *L. graveolens* Roxb., and two debatable species, *L. umbellata* M. Roem and *L. tuberose* Roxb., found in India (Prakash *et al*., 2013). *L. cylindrica* (Syn. *L. aegyptica*) and *L. acutangula*, are widely grown for their edible fruit and sponge-like fibers, whereas *L. echinata* and *L. graveolens* are wild species and known as bitter *Luffa*. Wild *Luffa* are having rough texture fruit with a very bitter flavor which are native to South Asia and Southeast Asia and grows abundantly in India, Bangladesh, Pakistan and Northern Tropical Africa with Gujarat, Bihar, Rajasthan and Madhya Pradesh being the most common locations in India (Mondal *et al*., 2022). Wild *Luffa* species are used in traditional Ayurvedic medicines, as they have various medicinal properties such as analgesic, laxative, antidepressant, anti-inflammatory, anxiolytic, antibacterial, antifungal, antiepileptic, hepatoprotective, antiulcer, anticancer, anti-anthelminthic and anti-hepatic, hence used to treat bronchitis, piles, jaundice and vaginal discharge (Prakash *et al*., 2013). Fruits, bitter in flavor and highly fibrous, are used to treat chronic bronchitis, dropsy, biliary, intestinal colic, putrid fever, and jaundice (Patel and Ghane, 2021). The whole plant is also used in curing leprosy, diabetes, antihelminthic, nephritis, rheumatism, stomachic, nephritis and abortifacient (Patel and Ghane, 2021).

Normally, seeds are equipped with suitable mechanisms to germinate under favorable environmental conditions. However, when a seed provided with adequate water, optimal temperature and sufficient oxygen for normal aerobic metabolism within physiological limits does not germinate, it is termed as "dormant" (Berrie, 1984). In plants, seed dormancy is a key evolutionary component, ensuring their survival in unfavorable conditions and allowing them to germinate when the chances of survival for the young seedlings are at the greatest (Koornneef *et al*., 2002). Seeds of wild *Luffa* species exhibit some degree of dormancy expressed by low or no germination. This may be attributed to hard seed coats that prevent water and oxygen from entering the seed, making it difficult for the embryo to germinate. The germination of the seeds of wild *Luffa* species has continued to be problematic and the seeds may require different scarification methods and exogenous treatments with chemicals to break dormancy and enhance germination, as have been reported for other species (Tambari and Aminu, 2015; Al-Menaie, 2010). Studies on pre-treatment methods to break dormancy in wild *Luffa* seeds have not received desired attention. This study, therefore, intends to identify the suitable pre-sowing treatments for breaking seed dormancy of freshly harvested seed of wild *Luffa* species *L. echinata* and *L. graveolens* (Accessions IC-587288, KC/CSR/ 41, KP/RS/PKS-20) in view of the economic, medicinal and agricultural potentials of these species.

Materials and Methods

Seeds of wild genotypes of *L. graveolens* and *L. echinata* (Table 1) used in this study were obtained from ICAR-National Bureau of Plant Genetic Resources, New Delhi. The present investigation was carried out at Division of Germplasm Conservation, ICAR-National Bureau of Plant Genetic Resources, New Delhi, during spring-summer and *Kharif* seasons of the year 2021-2022.

Luffa seeds (fresh and one year old seeds; 50 seeds of each) were subjected to different physical and chemical treatments for breaking seed dormancy (Table 2). A total of nine treatments (Table 2) were applied on the fresh seed of wild *Luffa* for breaking dormancy, whereas best treatments, including control were applied on one year old seed.

For seed germination studies standard germination test was conducted following ISTA rules (Anonymous, 2019). Other observations were recorded on ten random seedlings from each replication. Data on rate of seed germination was recorded regularly up to fourteen days and at the time of final count root and shoot length is taken with the help of a scale and expressed in "cm". Dry weight of ten random seedlings was recorded and further subjected to calculation of vigor indices. Seed vigor indices (SVI I and SVI II) were calculated using the following formula suggested by Abdul-Baki and Anderson (1973).

Vigor Index-I = Standard Germination (%) × Seedling length (cm)

Vigor Index-II = Standard Germination (%) × Seedling dry weight (g).

Germination percentage was calculated on the basis of fresh seeds, un-germinated fresh seeds and hard seeds on daily basis. The seedlings used for recording root: shoot ratio (RSR) were oven-dried at 70˚C for 48 hours after removing the cotyledon and seedling dry weight was expressed in gram per seedling as per the protocol of Kleyer *et al*. (2008). Germination rate was calculated as follows: Germination Rate = Σ(Gt/Dt), where Gt shows number of germinated seeds on day *'t'* and Dt is the number of days after placing seeds in germination paper when the germinated seeds were counted.

The experiment was conducted following CRBD, using three replicates, and subjected to ANOVA. ANOVA of each trait, including the mean, range, and coefficient of variation (CV%), were estimated using SPSS software 7.0. The data as percentage were transformed to arc sine values prior to statistical analysis.

Table 1: Source and identity of materials used in the experiment

Species	*Identity*	*Source*
L. graveolens	KP/RS/PKS/4 (IC-587288)	ICAR-NBPGR, New Delhi
	KC/CSR/ 41	
	KP/RS/PKS-20	
L. echinata	KP/SS/2871 (IC-618051)	

Table 2: Different treatment combinations used for breaking seed dormancy

S.N.	*Treatments (Fresh seed)*	*Treatments (One year old seed)*
1	Dry Seed	Dry Seed
2	Hydro-priming	Hydro-priming
3	GA_3 @ 200 ppm	Scarification
4	GA_3 @ 400 ppm	Scarification + GA_3 @ 200 ppm
5	KNO_3 @ 0.2%	Scarification + KNO_3 @ 0.2%
6	KNO_3 @ 0.4%	
7	Scarification	
8	Scarification + GA_3 @200 ppm	

Results and Discussion

In fresh seeds of *Luffa* genotypes germination percentage ranged from 0 to 88% (Figure 1) in nine different treatment combinations. Among all treatment combinations scarification + GA_3 @ 200 ppm was found superior to improve seed germination. In one year old dry *Luffa* seeds minimum germination of 36% was recorded in *L. echinata* which was improved up to 92% with scarification of seeds followed by chemical treatment with KNO_3 @ 0.2% (Figure 2). These findings are in line with Chaodumrikul *et al*. (2016) in *L. cylindrica* and Adhikari *et al*. (2021) in bitter gourd.

Germination percentage was negligible in freshly extracted wild *Luffa* seeds possibly due to higher levels of Abscisic Acid (ABA) which was lowered considerably on storage of seeds for one year or treatment of seeds either with GA_3/ KNO_3 (Hernández *et al*., 2022). In both fresh and one year old seeds of wild species *viz. L. echinata, L. graveolens* presence of hard seed coat hampered seed germination as its scrubbing resulted considerable improvement in seed germination. Hence, it can be interpreted that two types of dormancy exist in *Luffa i.e.* physical dormancy due to hard seed coat and another is physiological dormancy due to higher levels of dormancy inducing growth regulator. ABA is a key growth regulator known for its crucial role in dormancy induction in developing seeds or in maintenance of dormancy at time of seed imbibition, while gibberellic acid is widely documented for its key role in breaking of seed dormancy (Miransari and Smith, 2014). These interpretations are in line with those documented by Nambara *et al*., 2010 and Miransari and Smith, 2014.

In fresh *Luffa* seeds rate of seed germination varied between 0 to 6.88 (Table 3). Among all treatment combinations, scarification + GA3 @ 200 ppm significantly enhanced the seed germination rate over control (no treatment of seeds). Similarly, for one year old seeds rate of seed germination ranged from 1.8 to 5.48 (Table 4). Seed scarification followed by GA_3 treatment @ 200 ppm was found best for all three genotypes of *L. graveolens* included in this study. On the other hand, seeds of *L. echinata* genotype showed improvement in seed germination on exposure of its seeds to scarification, followed by chemical treatment with KNO_3 @ 0.2%. These findings are in line with Chaodumrikul *et al*. (2016) in *L. cylindrica*. Findings of this experiment pointed toward the presence of morphological barrier *i.e.* hard seed coat in wild *Luffa* spp. as scrubbing of hard seed coat with sandpaper showed considerable improvement in rate of seed germination. Seed dormancy is the most common adaptive mechanism prevalent in wild flora for survival under unfavorable environmental conditions (Baskin and Baskin, 2004). The beneficial effects of chemicals like KNO_3 were documented in bitter gourd (Renuga Devi and Jacqueline, 1995). Puncturing seed coat or scarification, seed treatment with KNO_3 is reported to reduce seed dormancy in cucurbits (Devi and Selvaraj, 1994).

For fresh seeds seedling length was recorded between 0 to 18.17 cm (Table 3). The control treatment showed no germination, while treatment of seeds with GA_3 @ 400 ppm recorded maximum seedling length in *L. echinata* genotype. GA_3 at higher concentration *i.e.* 400 ppm enhanced seedling length considerably but the seedlings became lanky and unfit for transplantation under open field conditions. Seedling length was recorded for one year old seeds between 10.84 and 15.40 cm (Table 4). Seed scarification followed by GA_3 treatment @ 200 ppm enhanced seedling length significantly over control and

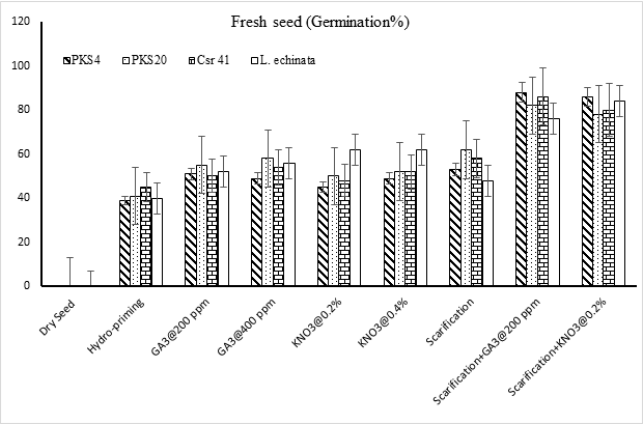


Figure 1: Effect of physical and chemical seed treatments on seed germination of fresh seeds of wild *Luffa* species

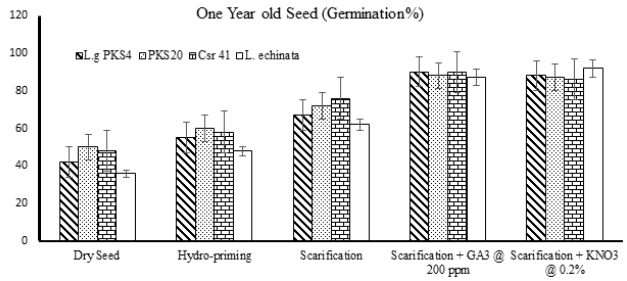


Figure 2: Effect of physical and chemical seed treatments on seed germination of one year old seeds of wild *Luffa* species

Table 3: Effect of seed dormancy breaking treatments (physical and chemical) on seed quality of freshly harvested seed of wild *Luffa* species

	Rate of seed germination				*Seedling length (cm)*				*Vigor index-I*				*Vigor index-II*			
Treatments	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*
Dry Seed	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00	0.00	0.00
Hydro-priming	1.5	2	1.33	0.96	9.48	10.02	9.46	6.88	369.72	410.82	425.7	275.2	0.55	0.74	0.92	0.81
GA_3 @ 200 ppm	2.25	3.41	3.48	1.44	12.38	12.32	11.92	10.35	631.38	677.6	596	538.2	0.78	1.19	1.47	1.16
GA_3 @ 400 ppm	2.28	3.81	3.52	1.42	15.38	15.52	14.91	18.17	753.62	900.16	805.14	1017.52	0.80	1.11	1.67	1.30
KNO_3 @ 0.2%	2.89	3.45	2.85	1.68	11.32	15.05	12.16	11.52	509.4	752.5	583.68	714.24	0.72	0.84	1.32	1.27
KNO_3 @ 0.4%	3.15	3.5	2.68	1.65	16.74	16.18	15.5	17.83	820.26	841.36	806	1105.46	0.84	0.82	1.48	1.49
Scarification	3.45	3.68	3.44	1.5	13.22	12.77	10.68	7.8	700.66	791.74	619.44	374.4	1.04	1.33	1.28	0.98
Scarification+GA_3@ 200 ppm	6.88	5.83	4.19	3.21	14.51	13.52	12.6	10.84	1276.88	1108.64	1083.6	823.84	1.85	1.84	2.34	1.79
Scarification+KNO_3@ 0.2%	4.33	5.77	3.58	3.6	13.99	13.8	11.5	11.85	1203.14	1076.4	920	995.4	1.64	1.67	2.03	2.03
CD @ 5%	0.6	0.51	0.48	0.62	1.12	1.38	0.98	1.02	81.2	77.6	70.8	84.7	0.01	0.02	0.02	0.01
CV	3.8	4.4	3.1	2.9	4.6	2.8	3.6	3	7.2	5.5	4.9	5.7	2.30	4.20	1.90	2.80

(A=*L.g*.KP/RS/PKS/4, B=*L.g*.KC/CSR/41, C=*L.g*.KP/RS/PKS-20, D=*L.e*.KP/SS/2871)

Table 4: Effect of seed dormancy breaking treatments (physical and chemical) on seed quality of one year old seed of wild *Luffa* species

	Rate of seed germination				*Seedling length (cm)*				*Vigor index-I*				*Vigor index-II*			
Treatments	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*
Dry Seed	2.55	2.4	1.8	2.2	11.54	12.56	12.2	10.84	484.68	628	585.6	390.24	0.66	0.81	0.88	0.69
Hydro-priming	3.85	3.2	2.2	2.5	12.58	12.88	13.42	11.92	691.9	772.8	778.36	572.16	0.92	1.05	1.09	1.11
Scarification	4.2	4.4	4.2	3.84	13.11	13.2	13.41	11.72	878.37	950.4	1019.16	726.64	1.20	1.24	1.49	1.33
Scarification + GA_3 @ 200 ppm	5.48	5.1	4.8	4.2	15.48	14.40	14.42	11.74	1386	1267.2	1297.8	1021.38	1.77	1.92	1.94	2.07
Scarification + KNO_3 @ 0.2%	5.18	4.8	4.84	4.4	15.21	14.32	13.94	12.84	1338.48	1245.84	1198.84	1181.28	1.72	1.74	1.70	2.30
CD @ 5%	0.57	0.69	0.72	0.49	1.44	0.89	1.16	1.09	67.5	71.5	56.8	65.1	0.01	0.02	0.02	0.01
CV	4.1	3.9	2.9	4.8	3.2	2.9	2.2	1.8	6.1	8.2	7.0	6.6	3.10	2.80	2.10	1.80

(A=*L.g*.KP/RS/PKS/4, B=*L.g*. KP/RS/PKS-20, C= *L.g*. KC/CSR/41, D=*L.e*.KP/SS/2871)

seedlings were uniformly healthy. These findings are in line with Tapfumaneyi *et al.* (2023) in Amaranthus. The major benefit of any physical or chemical seed treatment procedure is to increase germination and to ensure uniform seedling emergence. Overall, seed treatment to break seed dormancy leads to improved plant population and thus, higher productivity (Sridhar *et al*., 2013).

In fresh *Luffa* seeds vigor index-I valued between 0 to 1276.88 (Table 3) and for fresh seeds, seed vigor index-I for one year old seed was recorded between 390.24 to 1386 as shown in Table 4. The genotypes of *L. graveolens* exhibited a positive response to scarification followed by chemical treatment with GA_3 @ 200 ppm. In *L. echinata* seed scarification, chemical treatment with KNO_3 @ 0.2% was found most promising over other treatment combinations. These findings are in line with Tapfumaneyi *et al.* (2023) in Amaranthus. For fresh *Luffa* seeds values of seed vigor index-II were recorded between 0 to 2.34 (Table 3). In one year old seeds seed vigor index ranged from 0.66 to 2.30 (Table 4). Among nine different treatments, scarification followed by chemical treatment with either GA_3/KNO_3 was best suited in the enhancement of seed vigor indices and other growth parameters included in this study. These findings are in line with Tapfumaneyi *et al.* (2023) in Amaranthus.

The wild species of *Luffa* have hard seed coat and varied levels of physiological seed dormancy in freshly harvested and one-year-old seeds. Scarification and chemical seed priming can provide the necessary threshold to seed germination in all four accessions of wild *Luffa* spp. *viz. L. graveolens* and *L. echinata*. Seed germination treatments are quite helpful in improving seedling vigor and ensuring uniform crop stand immediately after seed extraction.

References

Abdul-Baki, A. A., & Anderson, J. O. (1973). Vigour determination of soybean seed by multiple criteria. *Crop Sci.*, **13**: 630–633.

Adhikari, B., Dhital, P. R., Ranabhat, S., & Poudel, H. (2021). Effect of seed hydro-priming durations on germination and seedling growth of bitter gourd (*Momordica charantia*). *PloS One*, **16(8)**: e0255258.

Al-Menaie, H. S., Al-Ragam, O., Al-Shatti, A., Mathew, M., & Suresh, N. (2010). The effects of different treatments on seed germination of the *Cassia fistula L*. and *Cassia nodosa* Buch.-Ham. Ex Roxb. in Kuwait. *Afr. J. Agric. Res*., **5(3)**: 230-235.

Anonymous (2019). International Rules for Seed Testing. The International Seed Testing Association, 8303 Barsserssorf, CH-Switzerland.

Baskin, J. M., & Baskin, C. C. (2004). A classification system for seed dormancy. *Seed Sci. Res*., ***14***: 1-16.

Berrie, A. M. M. (1984). Germination and dormancy. In: *Advanced Plant Physiology*. Pitams Publishing Limited. 128 Long Acre, London.

Chaodumrikul, S., Kaewsorn, P., Chulaka, P., & Chanprasert, W. (2016). Breaking seed dormancy in smooth loofah (*Luffa cylindrica* (L.) M. Roem.) using scarification and dry heat treatment. *J Agric Nat Resour*, ***50*(2)**: 85-88.

Devi, J. R., & Selvaraj, J. A. (1994). Effect of presowing treatment on germination and vigour in bitter gourd (*Momordica charantia* L.) cv. Co. 1. *Seed Res*., ***22***: 64-65.

Hernández, J. A., Díaz-Vivancos, P., Acosta-Motos, J. R., & Barba-Espín, G. (2022). Potassium nitrate treatment is associated with modulation of seed water uptake, antioxidative metabolism and phytohormone levels of pea seedlings. *Seeds*, ***1*(1)**: 5-15.

Kleyer, M., Bekker, R. M., Knevel, I. C., Bakker, J. P., Thompson, K., & Sonnenschein, M. (2008). The LEDA Traitbase: A database of life-history traits of the Northwest European flora. *J. Ecol*., ***96***: 1266–1274.

Koornneef, M., Bentsink, L., & Hilhorst, H. (2002). Seed dormancy and germination. *Curr. Opin. Plant Biol*., ***5*(1)**, 33-36.

Miransari, M., & Smith, D. L. (2014). Plant hormones and seed germination. *Environ. Exp. Bot.,* ***99***: 110–121.

Mondal, S., Bhar, K., Kumari, R., Mondal, P., Chakraborty, S., & Teja, N. Y. (2022). An insight into the elusive healer plant *Luffa echinata* Roxb. *Pharmacognosy Res*., ***15*(1)**, 26-41.

Nambara, E., Okamoto, M., Tatematsu, K., Yano, R., Seo, M., & Kamiya, Y. (2010). Abscisic acid and the control of seed dormancy and germination. *Seed Sci. Res.,* ***20***: 55–67.

Patel, S. B., & Ghane, S. G. (2021). Phyto-constituents profiling of *Luffa echinata* and in vitro assessment of antioxidant, anti-diabetic, anticancer and anti-acetylcholine esterase activities. *Saudi J. Biol. Sci*., ***28*(7)**: 3835-3846.

Prakash, K., Pandey, A., Radhamani, J., & Bisht, I. S. (2013). Morphological variability in cultivated and wild species of *Luffa* (Cucurbitaceae) from India. *Genet. Resour. Crop Evol*., ***60***: 2319-2329.

Renuga Devi, J., & Jacqueline, A. (1995). Seed pelleting and soil types on germination and vigour of seeds in ash gourd and ribbed gourd. *Madras Agric. J*., ***82*(2)**: 75-77.

Sridhar, S., Kumar, S. A., Thooyavathy, R. A., & Vijayalakshmi, K. (2013). Seed treatment techniques. Centre for Indian Knowledge Systems, Chennai Revitalising Rainfed Agriculture Network, 14. *http://www.ciks.org/6.SeedTreatmentTechniques.pdf*

Tambari, U., & Aminu, A. M. (2015). Effect of different seed treatment methods on the germination of *Senna obtusifolia* in Sudan Savanna ecosystem, Nigeria. *Am Educ Res J*., ***7*(2)**: 10-22.

Tapfumaneyi, L., Dube, P., Mavengahama, S., & Ngezimana, W. (2023). Effect of gibberellic acid and potassium nitrate seed treatments on the emergence and seedling vigor of amaranth and *Cleome gynandra*. *Agrosystems, Geosciences & Environment*, ***6*(1)**: e20359.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.13

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Effect of Nitrogen Nutrition Stress on Grain Weight, Starch Quality in Bread Wheat

Alka Bharati[1,2], Sachin Phogat[1], Jeet Roy[1], Amarjeet Kumar[3], Jayanthi Madhavan[4] and Pranab K. Mandal[1*]

Abstract

Grain weight in wheat is influenced by genetics and the environment, with nitrogen (N) nutrition playing a crucial role by improving grain filling and dry matter accumulation. Starch constitutes 60 to 70% of wheat grain dry matter, consisting of amylose and amylopectin. Amylose/amylopectin ratio determines the resistance nature of the starch and glycaemic index, which are important for human health. Nitrogen nutrition affects dry matter accumulation in wheat grain by influencing starch biosynthesis and enzymes involved in it. In this study, four wheat genotypes (HB-208, VL-829, chotilerma and HD-2967) with varied grain weights were grown under optimum nitrogen (ON) and low nitrogen (LN) conditions. The effect of nitrogen stress on grain weight; starch, amylose and amylopectin content were studied in these genotypes. Further, the important enzymes linked to amylose and amylopectin biosynthesis activity were also assayed in grains during the active grain-filling stages. HB-208 showed the highest grain weight, followed by VL-829, HD-2967 and chotilerma. Under nitrogen stress, grain weight was significantly reduced in HB (29%) and VL (25%), whereas no reduction was observed in case of HD and CL. Genotypic difference in grain filling was observed, where maximum starch accumulation in HD was observed before 21 days after anthesis (DAA) whereas, after 21 DAA in VL. We observed an increase in grain protein content under nitrogen stress in all three genotypes except HD. Among the two components of starch, amylose content was significantly reduced across the genotype under N stress, whereas amylopectin was unchanged in HB and VL and increased in HD and CL. Reduction in amylose resulted to starch reduction in HB and VL, which also corroborated with grain weight reduction. AGPase enzyme activity at 21 DAA was reduced in HB and VL, resulting in decreased starch content.

Keywords: Wheat, Nitrogen, Starch, Enzyme activity, Grain weight.

[1]Indian Council of Agricultural Research-National Institute for Plant Biotechnology, New Delhi, India

[2]Post Graduate School, Indian Council of Agricultural Research -Indian Agricultural Research Institute, New Delhi, India

[3]College of Agriculture, Central Agricultural University (I), Kyrdemkulai, Meghalaya, India

[4]Division of Genetics, Indian Council of Agricultural Research -Indian Agricultural Research Institute, New Delhi, India

***Author for correspondence:**

pranabkumarmandal@gmail.com

Received: 16/06/2023 **Revised:** 27/06/2023

Accepted: 27/06/2023

How to cite this article: Bharati, A., Phogat, S., Roy, J., Kumar, A., Madhavan, J., Mandal, P.K. (2023). Effect of Nitrogen Nutrition Stress on Grain Weight, Starch Quality in Bread Wheat. Indian J. Plant Genetic Resources. 36(3), 434-441.**DOI:** 10.61949/0976-1926.2023.v36i03.13

Introduction

Nitrogen nutrition is an indispensable factor for wheat production. During growth, especially during grain filling, nitrogen nutrition is a major limiting factor to wheat grain quality worldwide (Dupont and Altenbach, 2003). Studies show applying foliar nitrogen fertilizer during the later stages of wheat growth positively affects grain filling, particularly under drought conditions. Also, the omission of nitrogen fertilizer application in spring led to a significant reduction in both grain dry matter and grain nitrogen content by approximately 26 and 45%, respectively in winter wheat (Lv *et al.*, 2002; Blacklow and Incoll, 1981) and supplying nitrogen after flowering resulted in increased grain yield, (Madani *et al.*, 2010).

Wheat cultivars with different grain size exhibit variations in grain development. Endospermic cells are higher in larger grain type cultivar and take longer grain filling than small grain cultivar. Increased photosynthate production has been observed in larger gain type cultivars during mid grain grain-filling stage, providing ample substrate for grain development (Zhao *et al.*, 2003). Furthermore, enhanced conversion and utilization of assimilates in the sink tissues contribute to higher rates of starch accumulation

and filling in grains during the middle to late stages. The peaks of starch accumulation rate (SAR), photosynthetic rate, sucrose content, and activities of related enzymes in larger grain type typically occur during the middle to late filling stage and have a longer duration (Dai *et al*., 2009; Wang, 2014)

Starch, which constitutes 60–70% of the wheat grain weight, is the most vital carbohydrate in wheat grain. Around 20–30% of the starch is contributed by amylose, while the remaining 70–80% is attributed to amylopectin (Cornell, 2003). Amylose is a linear polysaccharide with glucose residues linked solely by α-1,4 glycosidic bonds. On the other hand, amylopectin is a branched molecule with branch points connected by α-1,6 glycosidic bonds, and α-1,4 glycosidic bonds connect the linear portions. Composition of starch, i.e., amylose and amylopectin ratio have profound effect on health. High amylose content increases the proportion of resistant starch in cereal grains. Resistant starch is the portion of starch that resists digestion in the small intestine. It is a type of dietary fibre offering various health benefits like improvement in insulin response, colon health and fullness (Slade *et al*., 2012). The amount and rate of accumulation of starch play a crucial role in determining the yield and quality of wheat. Starch synthesis primarily occurs in the endosperm cells by coordinated regulation of the series of enzymes (Ran *et al*., 2020). Notably, adenosine diphosphate glucose pyrophosphorylase (AGPase), soluble starch synthase (SS), granule-bound starch synthase (GBSS), and starch branching enzyme (SBE) have significant functions in starch synthesis. AGPase catalyzes the reaction between glucose-1-phosphate (G-1-P) and ATP, resulting in the formation of adenosine diphosphate glucose (ADPG) and pyrophosphoric acid (PPi) which is also a rate limiting step in starch biosynthesis hance, critical in determining the rate of starch synthesis and accumulation in the endosperm (Bowsher *et al*., 2007). SS and SBE is responsible for synthesizing amylopectin (Zhang *et al*., 2010; Zhang *et al*., 2011) and GBSS contribute to amylose synthesis by specifically binding to starch granules (He and Wei, 2020).

A study conducted on tartary buckwheat starch concluded that the activity of four key enzymes related to starch synthesis exhibited an initial increase followed by a decrease as nitrogen fertilization levels rose also, applying a moderate amount of nitrogen to rice crops resulted in a higher proportion of short amylopectin (DP 6–12), a lower proportion of long amylopectin (DP ≥ 37), an increased starch breakdown value, reduced SV (starch viscosity) and PT (pasting temperature) (Gao *et al*., 2021). Overall, nitrogen fertilization influences enzyme activity and substance accumulation, subsequently impacting crop quality. Relations between grain weight, starch content and enzymatic activity have been investigated in various abiotic stress, soil type and irrigation conditions (Zhang *et al*., 2021; Kim and Kim, 2021; Wang *et al*., 2008). however, there are very limited studies investigating its relation with nitrogen nutrition.

With the above background, while on one hand the nitrogen application needs to be reduced, the effect on starch quality of wheat grains, mainly the amylose and amylopectin content and their ratio is very important under low nitrogen regime. To address this important area, in the present study, four genotypes with diverse grain weight was grown under optimum and low soil nitrogen regime and the effect of nitrogen stress has been studied on grain filling, grain weight, amylose content and amylopectin content. In addition to the grain weight, important enzymes involved in starch biosynthesis were also investigated during mid grain filling period of developing grains.

Material and Methods

Plant Material and Growth Condition

A total of 4 genotypes were selected for the study having different grain weight and size at optimum N input. HB-208 (HB) had bold type grain, VL-829 (VL) and HD-2967 (HD) had medium size grain and chotilerma (CL) had small grain. Plants were grown on net house pots under two nitrogen treatments, 120 kg/ha (ON) having soil N content 150 kh/ha and 0 kg/ha (LN) having soil available nitrogen content 100 kg/ha. Experimental design, growth condition and soil N status are mentioned in Bharati *et al*., (2022). Grain dry weight was measured for a thousand grains in three replicates. Immature grains were harvested at 21 and 28 day after anthesis (DAA).

Starch Amylose and Amylopectin and Protein Estimation

Starch content was measured according to Kaufman *et al*., (2015). To measure starch content, freeze dried immature grains and dry mature grains were ground to powder. For 100 mg of powdered grain were extracted 3 times by boiling in 1-mL of 80% ethanol to remove soluble sugar. Pellet was dissolved in 1-mL of 95% DMSO by incubating it on boiling water bath for 1-hour with intermittent vortexing. After complete dissolution of starch, 10 µL of aliquot of dissolved starch was removed into fresh tube and mixed with 990 µL of water. In 200 µL of diluted sample was mixed with 10 µL of Lugol's reagent and absorbance was recorded at 595 nm. Standard curve was prepared from potato starch. Amylose content was determined according to Zhu *et al*., (2008) by dual wavelength iodine binding technique at 510 and 620 nm. Standard curve was prepared by potato amylose (A8515; Sigma–Aldrich). Amylopectin content was determined by subtracting amylose content to starch content. Total protein content was determined by measuring N content through Kjeldahl method followed by multiplying it with factor 6.2.

Enzyme Activity

100 mg grains were ground in 1-mL of extraction buffer containing 100 mM MOPS/NaOH (pH 7.2), 5 mM $MgCl_2$, 5% v/v glycerol, 5 mM dithiothreitol, 10 mg mL^{-1} BSA, 1%

(w/v) PVP. Samples were ground on ice cold mortar on ice followed by centrifugation at 10000 × g for 15 minutes at 4°C. Supernatant was collected for determining AGPase, UGPase, and SS activity. Pellet was again washed with 800 µL extraction buffer to remove any traces of SS. Pellets were suspended in 100 µL of extraction buffer for GBSS assay.

AGPase, UDPase and SS activity was assayed according to Nakamura *et al.*, (1989). For AGPase assay 40 µL of enzyme extract was mixed with 200 µL of reaction mixture containing 100 Mm HEPES-NaOH (pH 7.5), 3 Mm PPi, 4 mM Dithiothreitol, 1.2 mM ADP-Glucose and 5 mM $MgCl_2$. Mixture was incubated at 30°C for 20 minutes followed by placing in boiling water bath for 30 seconds to terminate the reaction. Mixture was centrifuged at 10000 × g for 10 minutes. To 200 µL supernatant 7 µL of 10 mM NADP was added and mixed well. Change in OD was recorded at 340 nm after addition of 1-µL of glucomutase (0.4 unit) and 1-µL of Glucose-6-phosphate dehydrogenase (0.35 unit).

UDPase assay was conducted in same manner as AGPase by only replacing ADP-Glucose to 1-mM UDP-Glucose in reaction mixture.

For assaying SS activity 20 µL of enzyme extract was combined with 120 µL reaction mixture (Mixture I) containing 50 mM HEPES-NaOH (pH 7.4), 0.7 mg amylopectin, 1.6 mM ADP-Glucose and 15 Mm dithiothreitol. Mixture was incubated at 30°C for 20 minutes followed by placing in boiling water bath for 30 seconds to terminate the reaction. To the 50 µL of solution containing 50 mM HEPES-NaOH (pH 7.4), 4 mM phosphoenol pyruvate, 10 mM $MgCl_2$, 200 mM KCl and 1.2-unit pyruvate kinase was added to mixture I and incubated at 30°C for 30 minutes followed by placing in boiling water bath for 30 seconds to terminate the reaction. Solution was centrifuged at 10000× g for 5 minutes and supernatant was collected (mixture II). 150 µL of mixture II was combined with 100 µL of solution containing 50 mM HEPES-NaOH (pH 7.4), 2 mM NADP, 20mM $MgCl_2$ and 10 mM glucose and incubated at 30°C for 10 minutes change in OD was measured after combining the above solution with 1-µL each of hexokinase (1.4 unit) and Glucose-6-phosphate dehydrogenase (0.35 unit).

GBSS activity was assayed according to Mukherjee *et al.,* 2015. Assay was conducted same as SS by using 40 µL of crude enzyme extract prepared from pellet and omitting amylopectin in 1st step.

Statistical Analysis

The standard error of means (SEM) for each mean value was calculated using standard methods using 3 replicates. Factorial analysis was performed using a completely randomized design (CRD) with treatment and genotypes as two factors to obtain ANOVA. The least significant difference at a significance level of 5% was calculated for the combination of treatment and genotype, and the range was determined through the range test. Values expressed using alphabetical symbols. The field plot layout followed a randomized block design (RBD).

Results

Grain Weight

Under ON, thousand grain weight (TGW) was highest in HB (44 g), followed by VL and HD (36.8 and 36.22). TGW was least in CL, and was significantly lower than other genotypes (27.22). Out of four genotypes, HB and VL showed significant reduction in grain weight under LN (27 and 25% reduction respectively). TGW of HD and CL remains unaffected under LN (Figure 1).

Starch Amylose and Amylopectin Content

Starch is principal component of grain dry matter of wheat, and hence, to understand starch biosynthesis and its two components viz. amylose and amylopectin is important. Starch amylose and amylopectin content was measured in immature (21 DAA, 28DAA) as well as mature grains (Table 1). In immature grains, starch content ranges from 332 to 616.2 mg. g^{-1} dry weight; whereas, in the mature grains this range was from 709 to 767 mg. g^{-1} dry weight. Under ON, HD had maximum starch at 21 DAA followed by HB, CL and VL. During 28 DAA starch content of HB, VL and HD became equivalent and CL showed significantly higher starch then other three genotypes. At maturity HB had maximum starch followed by VL and HD. Starch content of CL was least. Under LN all genotypes, except CL, exhibited a significant reduction in starch content at all growth stages. HB and VL experienced an approximate 3% reduction in starch content, while HD experienced a 2% reduction. Per grain reduction in starch was however drastic in HB and VL (25 and 29%). On the other hand, chotilerma demonstrated an increase in starch content at maturity. It is worth noting that the starch content in immature grains of CL was significantly lower under LN

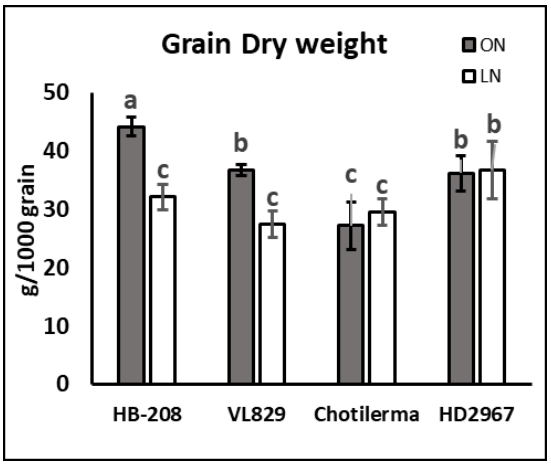


Figure 1: Grain weight per thousand grains under optimum and low nitrogen condition.

Table1: Starch, amylose, and amylopectin content at 21 and 28 DAA and maturity.

Component	*Growth stage*	*HB-208*		*VL-829*		*Chotilerma*		*HD-2967*	
		ON	*LN*	*ON*	*LN*	*ON*	*LN*	*ON*	*LN*
Starch (mg/g dry weight)	21 DAA	548.18 ± 0.99^{b}	332.37 ± 3.7^{f}	334.4 ± 1.46^{f}	210.5 ± 1.64^{g}	537.7 ± 1.05^{c}	403.4 ± 1.61^{e}	616.2 ± 1.66^{a}	516.5 ± 1.27^{d}
	28 DAA	557.7 ± 1.22^{b}	357.3 ± 2.89^{e}	557.2 ± 1.96^{b}	487.2 ± 3.10^{d}	565.8 ± 1.43^{a}	504.8 ± 1.80^{c}	552.0 ± 2.37^{b}	497.2 ± 1.43^{c}
	Mature	767.66 ± 2.6^{a}	738.67 ± 2.3^{c}	731.16 ± 2.1^{cd}	709.69 ± 4.8^{e}	712.13 ± 1.4^{e}	739.54 ± 3.1^{c}	754.39 ± 2.3^{b}	735.23 ± 0.7^{b}
Amylose (mg/g dry weight)	21 DAA	91.58 ± 2.43^{e}	79.37 ± 1.2^{f}	115.2 ± 2.02^{d}	33.3 ± 1.05^{g}	130.8 ± 3.34^{c}	163.3 ± 2.09^{d}	231 ± 4.37^{a}	164.9 ± 3.82^{b}
	28 DAA	123.43 ± 2.71^{e}	104.73 ± 3.8^{g}	114.67 ± 1.43^{f}	36.80 ± 0.7^{h}	137.51 ± 1.5^{c}	128.41 ± 2.2^{d}	261.82 ± 3.5^{a}	175.64 ± 2.7^{b}
	Mature	195.2 ± 0.58^{d}	169.53 ± 1.2^{f}	208.13 ± 0.24^{b}	191.6 ± 1.8^{e}	214.4 ± 0.95^{a}	203.66 ± 0.2^{c}	201.3 ± 0.31^{c}	193.6 ± 1.1^{de}
Amylopecti (mg/g dry weight)n	21 DAA	456.5 ± 3.4^{a}	252.9 ± 2.8^{d}	219.1 ± 4.2^{e}	177.1 ± 1.9^{f}	406.8 ± 2.9^{b}	240.03 ± 4.9^{d}	385.1 ± 3.4^{b}	351.5 ± 4.1^{c}
	28 DAA	434.2 ± 4.2^{ab}	252.5 ± 1.8^{e}	442.5 ± 3.2^{a}	450.3 ± 3.6^{a}	428.2 ± 2.8^{ab}	376.3 ± 2.2^{b}	290.1 ± 2^{d}	321.5 ± 1.9^{c}
	Mature	572.46 ± 4.7^{a}	569.14 ± 4.2^{a}	540.03 ± 3.2^{b}	501.6 ± 4.1^{bc}	497.73 ± 2.1^{c}	535.88 ± 3.9^{b}	553.09 ± 3.5^{a}	541.63 ± 2.9^{a}

conditions, suggesting an increase in the rate of starch synthesis after 28 DAA. Under ON, HD had highest amylose content in immature grain followed by CL, VL and HB. At maturity amylose content ranges from 195 to 214 mg. g^{-1} dry weight, with CL having the highest amylose content, followed by VL, HD, and HB. Throughout all growth stages, HB consistently had the lowest amylose content. Conversely, under LN conditions, all genotypes experienced a significant reduction in amylose, with HB experiencing the highest reduction of 13%, followed by VL with a reduction of 7%. At 21 and 28 DAA, HD exhibited the highest amylose content under both ON and LN conditions. Amylose content decreased as the grain matured, indicating that the maximum amylose synthesis occurred in HD at these stages. On the other hand, VL had relatively lower amylose compared to the other genotypes at 21 and 28 DAA. However, the amylose content significantly improved by maturity, at least under ON condition, suggesting that a substantial amount of amylose synthesis occurred after 28 DAA. Under ON condition, HB exhibited the highest amylopectin content at all growth stages. At 21 DAA, a reduction in amylopectin was observed under low nitrogen (LN) conditions in all genotypes. However, at 28 DAA, only HB and HD had significantly lower amylopectin content under LN compared to ON. In contrast to amylose, at maturity, HB compensated for amylopectin production and became on par with ON regime. VL also did not show any significant difference in amylopectin between the nitrogen treatments during maturity.

Amylose/amylopectin in mature grain ranges between 1: 2.3 to 1:2.9 under ON. Under LN condition range was found between 1:2.6 to 1:3.3 (Table 2). Under ON, CL had maximum amylose/amylopectin followed by VL, HD and HB in mature grains where difference between VL and HD was insignificant. Under LN condition HB and CL experienced decrease in amylose/amylopectin while changes in VL and HD was insignificant. Ratios was quit variable among genotypes throughout development phase. HB, VL and CL showed gradual increase in amylose/amylopectin whereas HD showed gradual decrease.

Protein Content

Protein content in the mature grain was found highest in HD followed by CL, VL and HD under ON. Under LN, all the genotypes except HD showed significant increase in on protein content whereas HD had significant reduction (Figure 2)

Enzyme Activity

In 4 key starch synthesizing enzymes adenosine diphosphate glucose pyrophosphorylase (AGPase), GBSS and soluble SS as well as uridine diphosphate glucose pyrophosphorylase (UGPase) were assayed at 21 and 28 DAA under ON and LN input to understand the role of enzyme in variation of starch, amylose, and amylopectin content under different nitrogen regime. All the enzyme activities were expressed as change in absorbance (OD) per minute per gram of fresh weight.

AGPase

AGPase is a rate limiting enzyme in starch biosynthesis pathway. AGPase activity in HB under ON was found to be highest at both the stages. Under LN, CL showed higher activity during 21 DAA. At 28 DAA VL and HD had highest activity under LN. At 21 DAA, only HB and VL showed a significant reduction in activity under LN, while at 28 DAA, all genotypes experienced a significant reduction. VL displayed a remarkable increase in AGPase activity at 28 DAA (9.4 and 7.6 min^{-1} g. f.wt.$^{-1}$ under ON and LN, respectively) compared to 21 DAA (5.6 and 4.9 min^{-1} g. f.wt.$^{-1}$ under ON and LN,

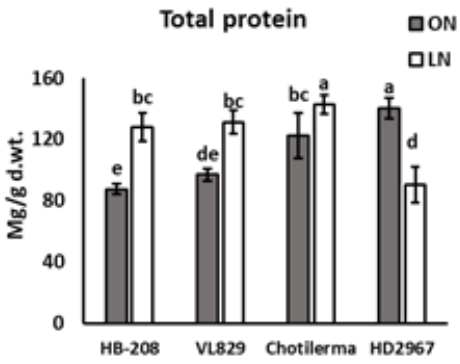


Figure 2: protein content in mature wheat grain under optimum and low nitrogen condition.

Table 2: Amylose/amylopectin ration in wheat grains

Genotype		*ON*			*LN*		
	Stage	*Amylose percentage*	*Amylopectin percentage*	*Ratio*	*Amylose percentage*	*Amylopectin percentage*	*Ratio*
HB-208	21 DAA	16.7^{e}	83.2^{a}	1: 4.9	23.8^{d}	76.11^{b}	1: 3.1
	28 DAA	22.1^{e}	77.8^{bc}	1: 3.5	29.31^{c}	70.68^{d}	1: 2.4
	Mature	25.4^{c}	74.5^{b}	1: 2.9	22.9^{d}	77.04^{a}	1: 3.3*
VL-829	21 DAA	34.4^{c}	84.1^{d}	1.9	15.82^{e}	84.17^{a}	1:5.3
	28 DAA	20.5^{e}	92.4^{b}	1:3.8	7.55^{f}	92.44^{a}	1:2.2
	Mature	27.8^{b}	72.3^{bc}	1:2.5	27.63^{b}	72.36^{c}	1:2.6
Chotilerma	21 DAA	24.3^{d}	59.5^{b}	1:3.1	40.48^{a}	59.51^{e}	1:1.4
	28 DAA	24.3^{d}	74.5^{cd}	1:3.1	25.44^{d}	74.55^{bcd}	1:2.9
	Mature	30.1^{a}	72.4^{d}	1:2.3	27.53^{b}	72.46^{c}	1:2.6*
HD-2967	21 DAA	37.4^{b}	68^{d}	1:1.6	31.93^{c}	68.06^{c}	1:2.1
	28 DAA	47.4^{a}	64.6^{f}	1:1.1	35.33^{b}	64.66^{e}	1:1.8
	Mature	26.4^{b}	73.6^{bc}	1:2.7	26.33^{b}	73.66^{bc}	1:2.7

*Significant difference between ON and LN at *p-value* 0.5

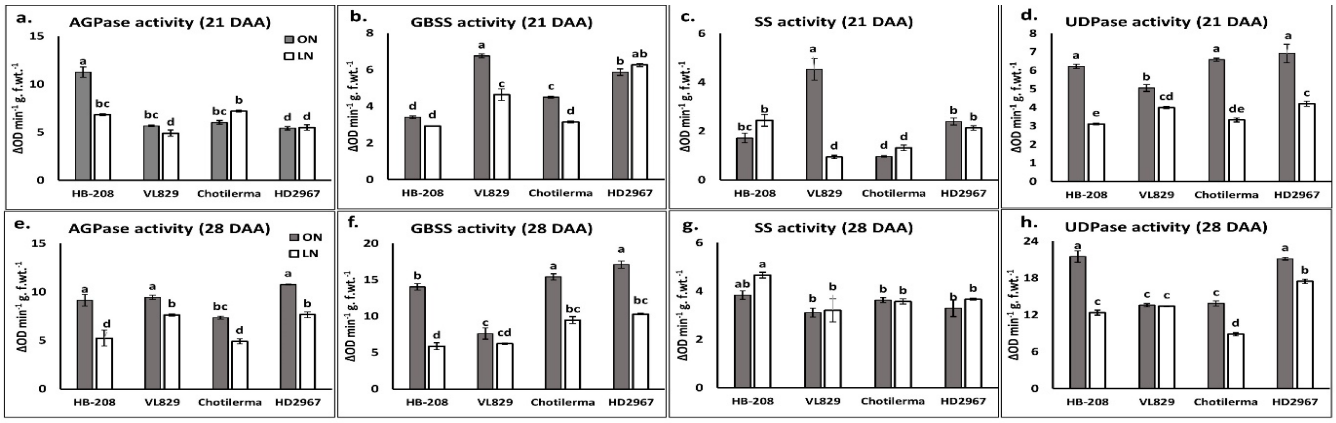


Figure 3: Activity of starch synthesizing enzyme at 21 and 28 DAA under optimum and low nitrogen condition.

respectively), providing an explanation for the increase in starch content following a similar pattern. The AGPase activity in HD under ON and LN was similar at 21 DAA but significantly reduced at 28 DAA under LN (Figure 3a, e).

GBSS

GBSS is the key enzyme determining amylose content in grains. GBSS activity ranges from 2.9 to 6.7 min^{-1} g. f.wt.$^{-1}$ during 21 DAA and 5.8 to 17 min^{-1} g. f.wt.$^{-1}$ during 28 DAA showing substantial increase in later growth period. At 21 DAA, the GBSS activity was similar between ON and low nitrogen LN conditions in HB and HD. However, VL and CL showed a significant decrease. A drastic reduction in GBSS activity was observed at 28 DAA under LN in HB, CL, and HD. In contrast, VL did not show a significant difference. HD consistently maintained significantly higher GBSS activity at both stages under ON, which explains its higher amylose content compared to other genotypes at these stages (Figure 3b, f).

SS

Like GBSS, an increase in SS activity was observed at 28 DAA compared to 21 DAA. The SS activity under both ON and LN conditions was on par in HB, CL, and HD at 21 DAA. Only VL exhibited a significant decrease in SS activity under LN. However, there was no significant difference in SS activity between N treatments at 28 DAA. Furthermore, at 28 DAA, HB displayed significantly higher SS activity compared to other genotypes. The SS activity in VL, CL, and HD was similar at 28 DAA (Figure 3c, g).

UGPase

UGPase is an important enzyme works in coordination with sucrose synthase and AGPase to maintain equimolar ration of ADP-glucose (precursor of starch synthesis) in cereal grains and hence plays a crucial role in determining starch content in grains. Notable increase in UGPase activity was observed at 28 from 21 DAA. HB and HD had highest activity

under ON. All the genotypes at 21 DAA and HB, CL, and HD at 28 DAA had significant reduction in UGPase activity under LN (Figure 3d, h).

Discussion

Grain weight accumulation in wheat is influenced by genetic traits such as grain filling rate and grain filling duration (Pržulj and Mladenov, 1999; Yu *et al*., 2022) as well as environmental factor like soil properties, temperature, soil nutrition status and N nutrition (Teng *et al*., 2023; Zheng *et al*., 2022; Dias and Lidon, 2009; Madani *et al*., 2010; Duan *et al*., 2018). Genotypes under investigation in this study exhibited variations in grain weight under different nitrogen regime. HB, characterized by bold seeds, displayed the highest TGW under optimum nitrogen (ON) conditions. However, under low nitrogen (LN), the TGW of this genotype decreased by 29%. Similarly, VL also experienced a reduction in grain weight by 25%. On the other hand, the TGW of the other two genotypes, CL, and HD, remained stable. Grain filling also varied among these genotypes both under ON and LN. at 21 DAA, grain filling was found maximum in HD (81%-ON to 70%-LN) and least in VL (45%-ON to 29%-LN). However, VL showed the maximum rate of starch accumulation from 21 DAA to maturity and HD showed least accumulation during this period. Therefore, genotypes like HD was efficient in grain filling at early stage of grain development, which has a relevance with climate change induced terminal heat stress; and it is likely that these genotypes will have less effect due to the stress (Shirdelmoghanloo, 2022). Studies has shown that N nutrition improves grain filling (Langer & Liew, 1973) and grain dry matter accumulation (Lv *et al.,* 2002; Blacklow and Incoll 1981). On the other hand, genotytpes with larger grain size do better photosynthesis during mid grain filling stage, which provides ample substrate for grain development (Zhao *et al*., 2003). Grain filling rate is an important determinant of final grain weight in various crops (Xie *el al*.,2015) and difference in final grain weight have been attributed to difference in grain filling rate in bread wheat (Xie *el al*.,2015) durum wheat (Gebeyehou *et al*., 1982) corn (Daynard *et al*., 1971) rice (Jones *et al*., 1979) and pear millet (Fussell *et al*., 1978)

In various abiotic stresses, a reduction in grain weight is often accompanied by a decrease in starch content and a reduction in the activity of starch metabolism enzymes (Zhang *et al*., 2010; Zhang *et al*., 2021). In our study, we observed both types of genotypes, one that displayed a reduction in grain weight and other one, which did not exhibit any significant change in grain weight under N-stress. This allowed us to investigate the reason with respect to accumulation of starch and its two components – amylose and amylopectin

Starch is the predominant component of wheat grains, accounting for around 60 to 70% of the grain's dry matter. Two types of alpha-glucan chain amylose and amylopectin forms starch in wheat. Among the two components of starch, amylose content was significantly reduced across the genotype. However, amylopectin content was unchanged in case of HB and VL, but increased in case of HD and CL. The ultimate effect was the reduction in total starch content in case of HB and VL, whereas no change in case of HD and CL. This is also corroborated with the grain weight reduction in case of HB and VL under LN condition. Previous studies also highlighted that various environmental stress as well as N input affects starch content in wheat grain (Kim and Kim., 2021; Asthir *et al*., 2017). These results not only highlight the quantity of starch and grain weight, but also exhibit the changes in starch quality (amylose: amylopectin ratio) under N stress condition. We observed, under LN conditions, all genotypes experienced a significant reduction in amylose, at all stages with HB and VL experiencing and highest reduction of (13 and 7%.) in mature grain. Amylose/amylopectin ratio in the mature grains is crucial parameter for starch quality determination. Starch with high amylose/amylopectin is beneficial for health (Slade *et al*., 2012). In our study, CL had the highest amylose/amylopectin ratio in the mature grain under ON conditions, while HB had the least. Under N stress condition, the amylose: amylopectin ratio in mature grain was significantly decreased in case of two genotypes, HB and CL.

Total grain protein in most of the genotypes were increased under N stress, that is mainly due to reduction in yield. The inverse relation between grain yield and grain protein content is already reported in wheat (Zörb *et al*., 2018).

Previous studies have indicated that the activities of enzymes involved in starch biosynthesis in wheat are influenced by both developmental factors and environmental conditions (Jenner *et al*., 1991; Hawker and Jenner, 1993; Jenner, 1994). It has been observed that the maximum activities of AGPase, starch synthase (SS) and granule-bound starch synthase (GBSS) in winter wheat grains are reached during the middle stage of grain filling (Xu *et al*., 2003). Hence, in our study, the activity of starch synthesizing enzymes was assayed at 21 and 28 DAA to investigate their role during these key developmental stages. AGPase, a rate-limiting enzyme in the starch biosynthesis pathway, responsible for catalyzing the first enzymatic step, which is the synthesis of ADP-glucose (Saripalli and Gupta, 2015), hence this enzyme is very important for both amylose and amylopectin biosynthesis. In our study the reduction in starch content as well as grain weight in HB and VL under N stress might be due the reduction in AGPase activity in these two genotypes at early grain filling stage (21 DAA). It is also reported that low AGPase activity is a major cause of low starch content in waxy wheat grains (Zi *et al*., 2018). GBSS enzyme, which is related to amylose biosynthesis (Zi *et al*., 2018), showed the reduction of its activity in all the genotypes, and probably the reason for low amylose

content under N-stress. Two other enzymes related to starch biosynthesis, SS and UDPase did not show any effect under N-stress.

Conclusion

Nitrogen stress not only affects yield and starch accumulation but also impacts starch quality. We observed a reduction in amylose content across all genotypes under N stress, while amylopectin content increased in some genotypes, maintaining the overall starch content. Consequently, the balance between amylose and amylopectin content determines whether the grain weight of a specific genotype will decrease or remain unchanged under N-stress condition. Our findings revealed that two of the genotypes, HB and VL experienced a decrease in both starch content and grain weight, primarily due to the reduction in amylose content. In the case of the HD and CL genotypes, the reduction in amylose was compensated by an increase in amylopectin content in the grain, resulting in no change in grain weight. Furthermore, we observed differential grain filling among the genotypes e.g., HD showed early grain filling and VL showed late grain filling under both ON and LN.

Acknowledgments

This work is a part of Ph.D. thesis to be submitted to ICAR-Indian Agricultural Research Institute, New Delhi. Authors would like to acknowledge the support and guidance provided by the PG School, Indian Agricultural Research Institute, New Delhi; Director, Indian Agricultural Research Institute, New Delhi; Director, ICAR-National Institute for Plant Biotechnology, New Delhi. This research is financially supported by the Department of Biotechnology, Govt. of India (BT/IN/UK-VNC/43/KV/2015-16) under the Indo-UK Centre for Improvement of Nitrogen Use Efficiency in Wheat (INEW) and ICAR- National Institute for Plant Biotechnology.

Conflicts of Interest

The authors declare that they have no conflict of interest

References

Asthir, B. D., Jain, Kaur, B., & Bain N. S. (2017) Effect of nitrogen on starch and protein content in grain influence of nitrogen doses on grain starch and protein accumulation in diversified wheat genotypes. *J. Environ. Biol.*, **38(3)**: 427.

Bharati, A., Tehlan, G., Nagar, C, K., Sinha, S, K., Venkatesh, K., & Mandal, P, K. (2022). Nitrogen stress-induced TaDof1 expression in diverse wheat genotypes and its relation with nitrogen use efficiency. *Cereal Res. Commun.*, **50**: 637–645

Blacklow, W. M., & Incoll, L. D. (1981) Nitrogen stress of winter wheat changed the determinants of yield and the distribution of nitrogen and total dry matter during grain filling. *Funct. Plant Biol.*, **8(2)**: 191-200.

Bowsher, C. G., Scrase-Field, E. F., Esposito, S., Emes, M. J., & Tetlow, I. J. (2007) Characterization of ADP-glucose transport across the cereal endosperm amyloplast envelope. *J. Exp. Bot.*, **58(6)**: 1321-1332.

Cornell, H. (2003) The chemistry and biochemistry of wheat. In: Cauvain SP (ed) Bread making: improving quality. Sawston: Woodhead Publishing Limited, 31-70.

Dai, Z., Yin, Y., & Wang, Z. (2009) Comparison of starch accumulation and enzyme activity in grains of wheat cultivars differing in kernel type. Plant growth regulation, **57**: 153-162.

Daynard, T. B., Tanner, J. W., & Duncan, W. G. (1971). Duration of the grain filling period and its relation to grain yield in corn, Zea mays L. 1. Crop Sci., **11(1)**: 45-48.

Dias, A. S., & Lidon, F. C. (2009) Evaluation of grain filling rate and duration in bread and durum wheat, under heat stress after anthesis. *J Agron Crop Sci.*, **195(2)**: 137-147.

Duan, J., Wu, Y., Zhou, Y., Ren, X., Shao, Y., Feng, W., & Guo, T. (2018) Grain number responses to pre-anthesis dry matter and nitrogen in improving wheat yield in the Huang-Huai Plain. Sci. Rep., **8(1)**: 1-10.

DuPon, F. M., & Altenbach, S. B. (2003) Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. J. Cereal Sci., **38(2)**: 133-146.

Fussell, L. K., & Pearson, C. J. (1978) Course of grain development and its relationship to black region appearance in Pennisetum americanum. Field Crops Res., **1**: 21-31.

Gebeyehou, G., Knott, D. R., & Baker, R. J. (1982) Rate and duration of grain filling in durum wheat cultivars 1. Crop Sci., **22(2)**: 337-340.

Hawker, J. S., & Jenner, C. F. (1993) High temperature affects the activity of enzymes in the committed pathway of starch synthesis in developing wheat endosperm. Funct. Plant Biol., **20(2)**: 197-209.

He, W., & Wei, C. (2020) A critical review on structural properties and formation mechanism of heterogeneous starch granules in cereal endosperm lacking starch branching enzyme. Food Hydrocolloids, 100:e105434.

Jenner, C. F. (1994) Starch synthesis in the kernel of wheat under high temperature conditions. Funct. Plant Biol., **21(6)**: 791-806.

Jenner, C. F., Ugalde, T. D., & Aspinall, D. (1991). The physiology of starch and protein deposition in the endosperm of wheat. Funct. Plant Biol., **18(3)**: 211-226.

Jones, D. B., Peterson, M. L., & Geng, S. (1979) Association Between Grain Filling Rate and Duration and Yield Components in Rice 1. Crop Sci., **19(5)**: 641-644.

Kaufman, R. C., Wilson, J. D., Bean, S. R., Herald, T. J., & Shi Y. C. (2015) Development of a 96-well plate iodine binding assay for amylose content determination. Carbohydrate polymers, **115**: 444-447.

Kim, K. H., & Kim J. Y. (2021) Understanding wheat starch metabolism in properties, environmental stress condition, and molecular approaches for value-added utilization. Plants, **10(11)**: 2282.

Langer, R. H. M., & Liew, F. K. Y. (1973) Effects of varying nitrogen supply at different stages of the reproductive phase on spikelet and grain production and on grain nitrogen in wheat. *Aust. J. Agric. Res.*, **24(5)**: 647-656.

Lv, X., Ding Y., Long, M., Liang, M., Gu, X., Liu, Y., & Wen X. (2021) Effect of foliar application of various nitrogen forms on starch accumulation and grain filling of wheat (Triticum aestivum L.) under drought stress. Frontiers in plant science, **12**: e645379.

Madani, A., Rad, A. S., Pazoki, A., Nourmohammadi, G., & Zarghami, R. (2010) Wheat (Triticum aestivum L.) grain filling and dry

matter partitioning responses to source: sink modifications under post anthesis water and nitrogen deficiency. Acta Scientiarum. Agron., **32**: 145-151.

Mukherjee, S., Liu, A., Deol, K. K., Kulichikhin, K., Stasolla, C., Brûlé-Babel, A., & Ayele B. T. (2015) Transcriptional coordination and abscisic acid mediated regulation of sucrose transport and sucrose-to-starch metabolism related genes during grain filling in wheat (*Triticum aestivum* L.). Plant Sci., **240**: 143-160.

Nakamura, Y. K., Yuki, S., Park Y., & Ohya, T. (1989) Carbohydrate metabolism in the developing endosperm of rice grains. *Plant and cell physiology*, **30(6)**: 833-839.

Pržulj, N., & Mladenov, N. (1999) Inheritance of

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.14

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Genetic Diversity Analysis of Bitter Gourd (*Momordica charantia* L.) Germplasm using Molecular Markers

Anupam Adarsh[1], Pankaj Kumar[2], Bishun D. Prasad[3], Randhir Kumar[4] and Hemant K. Singh[5*]

Abstract

Bitter gourd [*Momordica charantia* L.] is an important cucurbitaceous crop of medicinal and nutraceutical value. The present study was conducted to compare the efficacy of random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) molecular markers for genetic diversity analysis of bitter gourd. Twenty-one genotypes of bitter gourd, collected from different parts of India, were used for diversity analysis using two different types of markers. RAPD and ISSR markers yielded an average of 7.16 and 5.73 amplicon per primer, respectively. In the present study, RAPD and ISSR showed 89 and 63% polymorphic bands, respectively. Combined data analysis of RAPD and ISSR markers provided 73.66% polymorphism with 5.33 polymorphic amplicons per primer. The better discriminatory power of ISSR markers over RAPD markers may be due to comparatively higher values of average polymorphic information content (0.175) as well as the diverse nature of the genotypes. The information generated in this study would be helpful in designing breeding strategies for bitter gourd improvement.

Keywords: Bitter gourd, Cluster, Genetic diversity, Inter simple sequence repeats, Random amplified polymorphic DNA.

[1]Krishi Vigyan Kendra, Saraiya, Muzzafarpur, Bihar, India.

[2,3]Department of Molecular Biology & Genetic Engineering, Bihar Agricultural University, Bhagalpur, Bihar, India.

[4]Department of Horticulture (Vegetables & Floriculture), Bihar Agricultural University, Bhagalpur, Bihar, India.

[5]Farm Science Centre, Kishanganj, Bihar, India.

***Author for correspondence:**

horthemant16@gmail.com

Received: 10/08/2023 **Revised:** 05/09/2023

Accepted: 05/09/2023

How to cite this article: Adarsh, A., Kumar, P., Prasad, B.D., Kumar, R. and Singh, H.K. (2023). Genetic Diversity Analysis of Bitter Gourd (*Momordica charantia* L.) Germplasm using Molecular Markers. Indian J. Plant Genetic Resources. 36(3), 442-448. **DOI:** 10.61949/0976-1926.2023.v36i03.14

Introduction

Bitter gourd (*Momordica charantia* L.) is a flowering vine of important cucurbitaceous vegetables grown in the tropics and subtropics (Behera, 2004). It is considered an important vegetable with exceptional nutritional properties like high vitamin C and iron content (Singh *et al.*, 2013). It is a slender, climbing annual vine with long-stalked leaves and yellow, solitary male and female flowers borne in the leaf axils (Anilaumar *et al.*, 2015; Kumar and Khurana, 2016). *M. charantia* L. (Karela) is a flowering climber having actinomorphic and unisexual flowers (Mishra *et al.*, 2015). The fruit has a distinct warty look of exterior and an oblong shape (Gupta *et al.*, 2011). Bitter gourd is a monoecious crop with small yellow flowers with a high male-to-female ratio that varies from 9:1 to 48:1 (Thomas, 2008). This type of flowering behavior is undesirable because it results in poorer fruit set and yield, a common problem in bitter gourd cultivation (Adarsh *et al.*, 2019). To have a higher yield the ratio of staminate and pistillate flowers to be synchronized (Islam *et al.*, 2014). Androecy and gynoecycan usually be altered in bitter gourd by environmental factors such as temperature, photoperiod and nutrition (Dabholkar, 2006; Thomas, 2008). The bitter gourd plant parts possess a large number of medicinal properties and have been used as traditional medicine for diabetes as well as source of other health-related ailment treatment substances such as charantin (Yeh *et al.*, 2013; Grover and Yadav, 2004 and Dandawate *et al.* 2016).

Genetic diversity is the basic raw material for trait improvement in any crop species. Among the various forms of genetic resources, landraces play an important role in breeding for developing high-

yielding variety for both qualitative and quantitative traits where the crop's fruit yield depends on the variety's genetic potential (Dey *et al.*, 2007). Genetic diversity assessment based on the phenotypic characters is generally inaccurate, because environmental factors and plant developmental stages affect the morphological characters of the plant (Govindaraj *et al.*, 2015). Contrarily, the DNA markers-based diversity assessment is considered more accurate and unbiased since it is independent of stage of plant and tissue and also more reliable (Fan *et al.*, 2010; Saleh, 2011). Among the various DNA markers available, the random amplified polymorphic DNA (RAPD) has been widely used because it does not require prior sequence information of the genome and could be applied in any crop species. Further, this technique involves polymerase chain reaction (PCR) so very low quantity of DNA is needed for generation of markers. The RAPD technique provides for a quick way to survey polymorphism at a very large number of loci and has been used for genetic diversity analysis in a range of crop species, including bitter gourd (Dey *et al.*, 2007; Behera *et al.*, 2008; Collard *et al.*, 2005). Similarly, inter simple sequence repeats (ISSR) marker is also quite useful in detecting genetic polymorphism among genotypes as it can generate large number of markers that targeted multiple microsatellite loci distributed across the genome and has been used for genetic diversity studies in bitter gourd (Verma *et al.*, 2017; Pandey *et al.*, 2019). In the past few years, advancement in sequencing has enabled development of simple sequence repeat (SSR) markers in bitter gourd (Saxena *et al.*, 2015; Alhariri *et al.*, 2021; Cui *et al.*,2022). The SSR markers are locus-specific and thus amplification profile generated using these markers are highly reliable. The main limitation of this approach is that a large number of primers targeting various SSR motifs need to be synthesized and analyzed to find the polymorphic marker which is not affordable to laboratories with limited funding. In contrast to SSR marker-based analysis, RAPD and ISSR marker analyses are less expensive since the primers used in these approaches are generic, which implies that once the primers for these techniques are synthesized, they can be used to research genetic diversity in other species as well. Further, RAPD and ISSR are shown to reveal polymporhism more efficiently than SSR markers. The present study used RAPD and ISSR techniques because they are inexpensive and primers were already available in our laboratory.

In the present study, 21 diverse genotypes of bitter gourd from different locations of India were collected and screened for genetic diversity using RAPD and ISSR markers to identify useful germplasm for crop improvement in India for characterizing numerous undocumented indigenous bitter gourds.

Materials and Methods

Twenty-one bitter gourd genotypes used in this study (Table 1) were collected from different sources and grown and maintained at the vegetable research farm Bihar Agricultural University, Sabour, Bhagalpur, Bihar.

Table 1: Bitter gourd genotypes and their sources

Accession name	*Fruit color*	*Source*
Bitter Kathi	Dark green	VNR seeds
Line-114	Small light green	Sasaram collection
Line-214	Medium green	Madhubani collection
Line-314	Dark green	Sasaram collection
BRBTL	Light green	Local collection
Line-514	White	Madubani collection
Line-814	Light green	Muzaffarpur collection
Meghdoot	Small dark green	Nalanda collection
Preethi	Whitish green	KAU
Gangajali Small	Small light green	Nalanda collection
Jhalari	Medium green	Rasi seeds
Indira	Dark green	Seeds of India
Pusa Ausadhi	Medium green	IARI
Pirpaiti Local	Medium green	Pirpaiti collection
Konkan Tara	Medium green	BSKV Dapoli
Pusa Rasdar	Light green	IARI
BRBTW	Milky white	Local collection
Swarnayamini	Medium green	ICARNER, Patna
BRBTG	Light green	Sasaram collection
Leena	Green	VNR seeds
Pallee	Dark green	East West seeds

DNA Isolation

The genomic DNA from young, healthy leaves was extracted using the cetyl trimethyl ammonium bromide (CTAB) method described previously (Anu *et al.*, 2015).

Marker Analysis

RAPD analysis was carried out with 10 decamer random primers procured from Operon Technologies, California, USA. RAPD analysis was performed in 25 µL volume containing 1X Taq DNA polymerase buffer, 200 µM dNTPs mixture, 0.5 µM primer, 25 ng of template DNA and 1 U of Taq DNA polymerase (Bangalore Genei, Bangalore, India) in a thermal cycler (Eppendorf™ Nexux, Germany). The PCR conditions included initial denaturation DNA at 94°C for 4 minutes; followed by a 45-cycle amplification (94°C, 1-minute; 38°C, 1-minute; 72°C, 2 minutes) and a final extension step at 72°C for 7 minutes.

For ISSR analysis also, primers were obtained from Operon Technologies, California, USA. ISSR amplification was performed in 25 µL volume containing 1X Taq DNA polymerase buffer, 200 µM dNTPs mixture, 0.5 µM primer, 25 ng of template DNA and 1 U of Taq DNA polymerase (Bangalore Genei, Bangalore, India) in a thermal cycler performed in 25 µL volume containing 1X Taq DNA polymerase. The reagents were mixed thoroughly and then placed on a thermal cycler for cyclic amplification and the conditions for amplification. DNA denaturation was done at 94.8°C for 5 minutes, followed

by a 40 cycle amplification (94°C for 1-minute; 36°C for minutes; 72°C for 2 minutes) followed by last step of final extension at 72°C for 5 minutes. Amplification products were then subjected to electrophoresis in 1.2% agarose gel using 1X TBE and detected by ethidium bromide staining, viewed under ultraviolet light and photographed with a gel-documentation system.

Data Analysis

NTSYS-pc Version 2.02 (Numerical Taxonomic System) software (Rohlf, 2000) was used to calculate Jaccard's similarity coefficients between genotypes. The similarity matrix was subjected to the cluster analysis of the unweighted paired group method using arithmetic averages (UPGMA) and dendrograms were generated. The polymorphic information content (PIC) was estimated by using the formula PIC = 2F (1-F), where F is the frequency of band present (Lynch and Walsh, 1998).

Results

Identification and Evaluation of RAPD & ISSR Markers for Genetic Diversity Analysis

A total of 15 ISSR and 25 RAPD markers were tested for amplification in bitter gourd genotypes. Eleven ISSR and 18 RAPD markers yielded reproducible and scorable amplified products in all the genotypes of bitter gourd studied. A list of ISSR and RAPD markers used in this study is shown in Tables 2 and 3.

RAPD Analysis

Out of 129 reproducible amplicons generated using 18 RAPD primers in 21 bitter gourd genotypes, 89% were polymorphic (Table 1). The size of the amplified products varies from approximately 100 to 1000 bp. The number of amplicons per primer ranged from four (OPA-4, OPB-8) to eleven (OPA-10, OPA-19), averaging 7.167 amplicons. The number of polymorphic amplicons per primer ranges from two (OPA-4) to nine (OPA-19 and OPC-14) with an average of 4.94. The percentage of polymorphism ranged from 42.86% (OPB-6 and OPC-10) to 90.00% (OPC-14), with an average of 68.001% (Table 1). The average PIC value was 0.160 and ranged from 0.043 (OPC-8) to 0.277 (OPA-12) (Table 1). The Jaccard's similarity coefficients of 21 bitter gourd genotypes based on 18 RAPD markers were computed. The similarity coefficients ranged from 0.52 to 0.93 with an average correlation coefficient of 0.72 (Figure 1).

ISSR Analysis

The size of the amplified products varies from ~100 bp to 1200 bp (Table 2). The number of amplicons per primer ranged from four (ISSR-51) to twelve (ISSR-8) with

Table 2: Banding pattern and statistics of RAPD primers of 21 genotype

S. No.	*Primer (Operon code)*	*Sequences*	*Total no. of amplified fragments*	*Monomorphic bands*	*Polymorphic bands*	*Range of amplicon size (bp)*	*Polymorphism (%)*	*PIC*
1	OPA-4	AATCGGGCTG	4	2	2	350–1500	50.00	0.067
2	OPA-5	AGGGGTCTTG	7	2	5	350–1500	71.43	0.212
3	OPA-8	GTGACGTAGG	5	1	4	300–1000	80.00	0.084
4	OPA-9	GGGTAACGCC	6	3	3	250–1000	50.00	0.243
5	OPA-10	GTGATCGCAG	11	5	6	300–2000	54.54	0.101
6	OPA-12	TCGGCGATAG	7	3	4	250–1000	57.14	0.277
7	OPA-15	TTCCGAACCC	6	2	4	300–2000	66.67	0.132
8	OPA-17	GACCGCTTGT	8	1	7	300–1800	87.50	0.162
9	OPA-18	AGGTGACCGT	7	2	5	300–1500	71.43	0.154
10	OPA-19	CAAACGTCGG	11	2	9	250–1000	81.82	0.231
11	OPB-1	GTTTCGCTCC	9	2	7	300–1500	77.78	0.222
12	OPB-6	TGCTCTGCCC	7	4	3	250–1200	42.86	0.144
13	OPB-8	GTCCACACGG	4	1	3	250–1300	75.00	0.131
14	OPB-12	CCTTGACGCA	6	1	5	300–1400	83.33	0.125
15	OPC-8	TGGACCGGTG	8	2	6	300–1200	75.00	0.043
16	OPC-10	TGTCTGGGTG	7	4	3	250–1200	42.86	0.206
17	OPC-14	TGCGTGCTTG	10	1	9	300–1200	90.00	0.202
18	OPC-15	GACGGATCAG	6	2	4	300–1000	66.67	0.146
	Total		129	40	89			
	Average		7.167	2.222	4.944		68.001	0.160

Table 3: Banding pattern and statistics of ISSR primers of 21 genotype

S. No.	*Primer (Operon code)*	*Sequences*	*Total no. of amplified fragments*	*Monomorphic bands*	*Polymorphic bands*	*Range of amplicon size (bp)*	*Polymorphism (%)*	*PIC*
1	ISSR4	ACACACACACACACACAG	6	1	5	250–800	83.33	0.138
2	ISSR8	ATGATGATGATGATGATG	12	2	10	300–1200	83.33	0.208
3	ISSR9	GAGGAGGAGGAGGAGGAG	5	1	4	300–2000	80.00	0.240
4	ISSR22	ACACACACACACACACAA	11	2	9	250–1000	81.82	0.134
5	ISSR23	TGTGTGTGTGTGTGTGAT	8	1	7	350–1300	87.50	0.159
6	ISSR24	TGTGTGTGTGTGTGTGAG	8	3	5	300–1500	62.50	0.221
7	ISSR35	AGAGAGAGAGAGAGAGGTA	6	1	5	250–1000	83.33	0.159
8	ISSR42	TGTGTGTGTGTGTGTGGC	6	1	5	250–1200	83.33	0.168
9	ISSR43	ACACACACACACACACCG	7	1	6	280–1100	85.71	0.254
10	ISSR44	ACACACACACACACACGA	6	2	4	260–1200	66.67	0.236
11	ISSR51	TGTGTGTGTGTGTGTGAT	4	1	3	250–1500	75.00	0.125
Total			79	16	63			
Average			7.18	1.45	5.73		79.32	0.190

an average of 7.18 amplicons per primer (Table 2). The average number of polymorphic amplicons per primer was 5.73, ranging from three (ISSR-51) to ten (ISSR-8). The percentage of polymorphism ranged from 62.50 (ISSR24) to 85.71% (ISSR-43), with an average of 79.32%. The maximum number of polymorphic amplicons was obtained with the primers ISSR8 (10), ISSR22 (9) with an average of 5.73 per primer. The average PIC value was 0.19 and ranged from 0.125 (ISSR-51) to 0.254 (ISSR-43). In all the primers, ISSR-43, ISSR-44, ISSR-9, and ISSR-8 had higher PIC values. The Jaccard's similarity coefficients of 21 bitter gourd lines based on 11 ISSR markers were computed (Figure 2). The similarity coefficients ranged from 0.52 to 0.91, with an average correlation coefficient of 0.71.

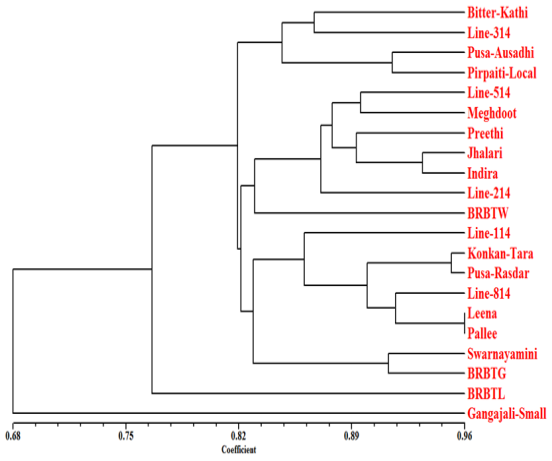


Figure 1: Dendrogram based on RAPD markers using Jaccard coefficient

Polymorphism Analysis using RAPD and ISSR Markers

Out of 208 reproducible amplicons generated by RAPD and ISSR primers in combination, 152 were found to be polymorphic. The average amplicons per primer were 7.173 of which 5.335 were polymorphic. The average percentage of polymorphism was 73.661. The average value of PIC, was 0.175, respectively. The Jaccard's similarity coefficients for combinations of 21 bitter gourd lines based on combined data analysis was computed (Figure 3). The coefficients ranged from 0.50 to 0.93.

Cluster Analysis Based on RAPD Analysis

The phylogenetic tree of the relationship among the 21 bitter gourd lines using 18 RAPD primers was shown in Figure 1. All genotypes were grouped in five clusters, namely, I (similarity coefficient = 0.52), II (similarity coefficient = 0.62), III (similarity coefficient = 0.72), IV (similarity coefficient = 0.82), and V (similarity coefficient = 0.93). The maximum number of genotypes was divided into two clusters, I and II. Cluster I was divided into two sub-cluster I and II. The sub-cluster was divided into three categories i.e. A1, A2, and A3. The category A-2 contains a large number of genotypes, followed by A-3 and A-1. The category A-2 was divided into two sub-categories A-1-1 and A-2-1. The category A-2-1 contains line-514, Meghdoot, Preethi, Jhalari, Indira and line-214, whereas A-2-2 contains BRBTW. Category A-3-1 contained line -814, Konkan Tara, Pusa rasdar, Leena and Pallee whereas category A-3-2 contained swarnayamini and BRBTG. The category A-1-1 contains bitter Kathi, and Line-314, whereas A-1-2 contains Pusa Aushadhi and Pirpaiti local. The sub-cluster II contained one genotype *i.e.* Gangajalee Small. The association amongst different genotypes was presented as a dendrogram. The resemblance coefficient

between the two genotypes is the value at which their branches join. The dendrogram also showed the relative magnitude of resemblance among different clusters.

Cluster Analysis Based on ISSR Analysis

The dendrogram generated from the Jaccard's similarity values using NTSYS software based on 11 ISSR primers presented in Figure 2. The genotypes were grouped into clusters I and II with a similarity coefficient (0.71). Cluster I was divided into two sub-cluster I, II. The sub-cluster I of cluster I was divided into Cat-A and Cat-B. The Cat –A contained bitter kathi and line-514. The Cat-B contained in Preethi, Gangajalee small, Pusa Aushadhi, Jhalari, Indra and Pirpaiti local. Sub-cluster-II was divided into Cat-A and Cat-B. Cat-A contained BRBTL and line -314, whereas Cat-B contained meghdoot and line-214. Cluster II was divided into sub-cluster-I -I and sub-cluster-II. Subcluster I was divided into Cat-A and Cat-B in which Cat-A contained line-814, Konkan Tara, Pusa Rasdar and BRBTW whereas Cat-B contained Leena and Palee. The sub-cluster –II had only one category, i.e., Cat-A. It contained Swarnayamini and BRBTG. The dendrogram also showed the relative magnitude of resemblance among different clusters.

Cluster Analysis Based on RAPD and ISSR Analysis

The dendrogram generated by combined RAPD and ISSR marker analysis (Figure 3) categories the genotypes into five clusters, I (similarity coefficient = 0.53), II (similarity coefficient = 0.63), III (similarity coefficient = 0.72), IV (similarity coefficient = 0.82) and V (similarity coefficient = 0.92). All the lines were divided into two clusters. Cluster I contained one line *i.e.* gangajalee small and cluster II were divided into two sub-cluster. Sub-cluster I contains only one line, *i.e.,* line-414 (BRBTL) while sub-cluster II was divided into two categories. Categories A contained were divided into sub-categories. Categories IA contained bitter kathi and Line-314, whereas categories IB contained Line-514, Meghdoot, line -214, Preethi, Pusa Aushadhi, Pirpaiti local, Jhalari and Indira. The categories IIA contained line-814, Konkan Tara, Pusa Rasdar, Leena and Pallee, whereas line II B contained Karela Safed (BRBTW), Swarnayamini and BRBTG.

Discussion

Genetic diversity is a pre-requisite for any crop improvement program to assess the variation among the plant's genotypes, genes or whole genomes. Genetic characterization of different bitter gourd genotypes provides an opportunity to broaden the genetic base of bitter gourd (Behera *et al.*, 2008). In the present study, the average percentage of polymorphism obtained by RAPD markers was 68.00 which was higher than 41.34% (Dalamu *et al.*, 2012), 36.5% (Dey *et al.*, 2006) and 48.3% (Rathod *et al.*, 2008).

However, percentage polymorphism (79.32%) revealed by ISSR markers was higher than those obtained by Dey *et al.* (2006) in bitter gourd (36.50%) Verma *et al.* (2007) in ash gourd (>80%), Behera *et al.* (2008) in bitter gourd (74.7%) and Dalamu *et al.* (2012) in bitter gourd (70.0%). The average number of polymorphic amplicons per RAPD primer was 4.94 and 5.35 in ISSR primers. However, Dalamu *et al.* (2012) obtained a higher number of average polymorphic amplicons per primer, 3.72 amplicons per ISSR primer whereas Behera *et al.* (2008) obtained 6.3 amplicons per primer.

The relative efficiency of marker types for genetic analysis varies among crop species. The present study concluded that ISSR markers were more effective than RAPD analysis. The better discriminatory power of ISSR markers over RAPD markers may be due to comparatively higher values of average PIC; 0.190 (ISSR) versus 0.160 (RAPD).

In our study, ISSR marker-based analysis of genetic diversity of bitter gourd genotypes revealed greater diversity when compared with RAPD and ISSR analyses in previous studies with different sets of bitter gourd germplasm [RAPD (36.5% polymorphism); Dey *et al.* (2006) and ISSR (74.5% polymorphism); Singh *et al.*, 2015]. In the present study, the average number of polymorphic bands was 4.94 per RAPD primer and 5.35 per ISSR primer. The values for polymorphism per primer in the similar range have been reported by Dey *et al.*, (2006). [RAPD (2.62) and ISSR (6.30)]. Similar results for RAPD and ISSR polymorphism was observed in relation to other crops like watermelon (Paris *et al.*, 2003), and watermelon *Cucumis lanatus* (Levi *et al.*, 2004). When compared to other arbitrary primers like

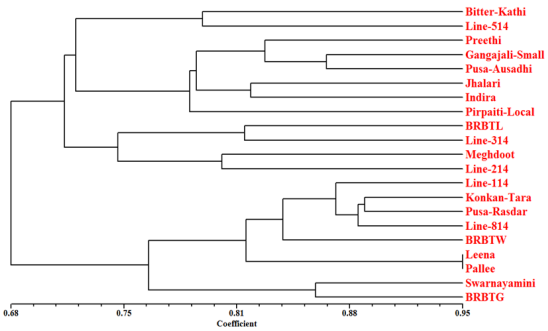


Figure 2: Dendrograms based on ISSR primers

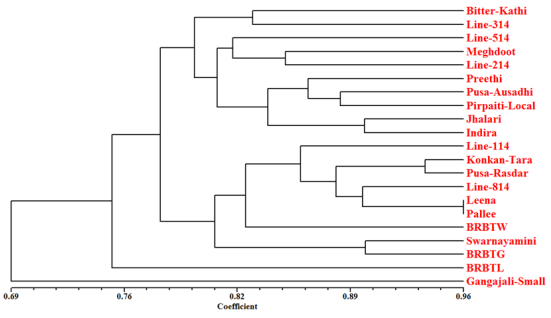


Figure 3: Dendrogram generated by combined 18 RAPD and 11 ISSR primers

RAPDs, ISSRs offer enormous potential for resolving intra- and inter-genomic relationships (Zietkiewicz *et al.*, 1994) and genus-specific bands, thus in an earlier study, this marker system was utilized for defining the uniqueness and genetic-relatedness among various cucurbitaceous members (Payel *et al.*, 2015). The increased level of diversity observed in our study was likely due in part to the number and comparatively high discriminatory power of ISSR markers employed and the diverse nature of the germplasm examined.

The Jaccard's similarity coefficient values for RAPD, ISSR and combined RAPD and ISSR data showed a wide range, suggesting that the bitter gourd germplasm collection represents a genetically diverse population. The similar range of Jaccard's similarity coefficient values for RAPD (0.72–0.97) and ISSR (0.50–0.95) markers were reported on previous study as well (Dalamu, 2011). Similarly, in another study, Behera *et al*. (2008) reported Jaccard's similarity coefficient values ranging between 0.57 to 0.93 for RAPD and between. 48 to 0.91 for ISSR were observed for the bitter gourd accessions that they had studied.

The genotypes lying closer to each other in dendrograms were more similar to those lying apart. The dendrogram showed the relative magnitude of resemblance among different clusters. In the UPGMA dendrogram drawn combined RAPD and ISSR data, genotypes from different sources were also clustered together, which may be attributed to sharing germplasm lines among the public sector institutions. The two genotypes (Leena and Pallee) from the private seed companies could not be distinguished even in the dendrogram drawn using combined RAPD and ISSR. Further, the dendrogram and genetic similarity matrix produced from marker's data was compared and revealed similar but not identical phylogenetic relationships. The results obtained from these two marker systems were in agreement. Based on DNA profiling using ISSR and RAPD PCR analysis, similar magnitudes of correlation were also found by Dalamu *et al.* (2011) and Behera *et al.* (2008) in bitter gourd and Paris *et al*. (2002) in *Cucurbita pepo*.

When RAPD, ISSR, and RAPD + ISSR derived dendrograms were compared, the discrimination among genotypes within the clusters was more effective than using them separately. The most of the accessions studied in the present experiment have differed with their geographic origin. Eastern India has been proposed as one of the centers for the domestication of bitter gourd (Behera *et al.*, 2008; Yang and Walters, 1992).

Acknowledgment

The authors are grateful to the Vice Chancellor BAU Sabour, Chairman of Horticulture (Vegetable & Floriculture) and Chairman of MBGE lab of BAU Sabour for providing the laboratory facilities and financial support for conducting the research.

Funding

This work was supported by Bihar Agricultural University, Sabour, Bhagalpur, Bihar India (D/HORT/26/2014-15). This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The financial support was provided by the Department of Horticulture (Vegetable & Floriculture), and the Department of Molecular Biology and Genetic Engineering, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India for conducting research by providing lab, basic facilities for work on molecular marker (RAPD and ISSR primer).

References

Adarsh,A.,Kumar,R.,Bhardwaj,A. & Chaudhary,H.C. (2019). Correlation matrix study in bitter gourd for qualitative and quantitative traits. *J. Pharmacogn. Phytochem.* **8**: 3023–3027.

Alhariri, A., Behera,T.K., Jat,G.S., Devi,M.B., Boopalakrishnan, G., Hemeda,N.F., Teleb,A.A., Ismail,E.& Elkordy, A,.(2021). Analysis of Genetic Diversity and Population Structure in Bitter Gourd (*Momordica charantia* L.) Using Morphological and SSR Markers. *Plants*. 2021; **10(9)**: 1860. *https:// doi.org /10. 3390/ plants10091860.*

Anu,A.,Prasad,B., Kumar,R., Kumar,P., Patel,V.B. & Jha,R.N. (2015). Clonal variability studies in 'langra' mango (Mangifera indica L.) using morphological, biochemical and molecular markers. Int. *J. of Agri. Env and Biotech.* **8(3)**: 567-581.

Anilakumar,K.R.,Kumar,G.P.&Haiyaraja,N.(2015).Nutritional, pharmacological and medicinal properties of *Momordica charantia*. *Int. J. Nutr. Food Sci.* **4**: 75–83.

Behera, T.K. (2004). Heterosis in bitter gourd. *J. New Seeds* **6**: 217–222.

Behera, T.K., Gaikward, A.B., Singh,A.K. & Staub,E. (2008). Relative efficiency of DNA markers (RAPD, ISSR and AFLP) in detecting genetic diversity of bitter gourd (*Momordica charantia* L.). *J.Sci. Food Agric.* **88**: 733–737.

Collard,B.C.Y., Jahufer,M.Z.Z., Brouwer,J.B. & Pang,E.C.K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts." *Euphytica* **142**: 169–196.

Cui,J., Cheng, J., Nong,D., Peng,J., Hu, Y., He, W., Zhou, Q., Dhillon, N.P.S. & Hu,K.(2017).Genome-wide analysis of simple sequence repeats in bitter gourd (*Momordica charantia*). *Front. Plant Sci.* **8**: 1103.

Cui,J., Zhou, Y., Zhong,J., Feng, C., Hong, Y.,& Hu, K., (2022). Genetic diversity among a collection of bitter gourd (*Momordica charantia* L.) cultivars. *Genet Resour Crop Evol* **69**: 729–735. Dabholkar AR (2006) General Plant Breeding. A.K. Mittal Publisher,Mohan Garden, India.

Dalamu (2011). Genetic diversity analysis and inheritance of fruit traits in bitter gourd (*Momordica charantia* L.). PhD thesis, *Ind Agril Res Inst*., New Delhi.

Dalamu, Behera,T.K., Gaikwad,A.B., Saxena,S., Bharadwaj, C. & Munshi,A.D.(2012) Morphological and molecular analyses define the genetic diversity of Asian bitter gourd (*Momordica charantia* L.). *Aust. J. Crop Sci.* **6**: 261–267.

Dandawate, P.R., Subramaniam,D., Padhye,S.B. & Anant,S. (2016). .Bitter melon: a panacea for inflammation and cancer. *Chin. J. Nat. Med.* **14**: 81–100.

Dey,S.S., Behera,T.K., Munshi,A.D. & Sirohi,P.S.(2007). Studies on

genetic divergence in bitter gourd (*Momordica charantia* L.). *Indian J. Hort*. **64**: 53–57.

Dey, S.S., Singh, A.K., Chandel, B. & Behara, T.K. (2006). Genetic diversity of bitter gourd (*Momordica charantia* L.) genotypes revealed by RAPD markers and agronomic traits. *Sci. Hort*. **109(1)**: 21-28.

Esmailnia,E., Arefrad, Mehdi, Shabani,S., Mohammadreza, Vafadar. F.& Dehestan,A.(2015).Genetic diversity and phylogenetic relationship of Iranian indigenous cucurbits investigated by Inter Simple Sequence Repeat (ISSR) markers. **9(1)**: 47-54.

Fan, C., Cai, G.,Qin, J., Li,Q., Yang,M., Wu,J., Fu, T., Liu, K. & Zhou, Y.(2010). Mapping of quantitative trait loci and development of allele- specific markers for seed weight in *Brassica napus*. *Theor. Appl. Genet*. **121**: 1289–1301.

Gajera,B.B., Kumar,A., Singh,A.S., Punvara,B.S., Ravikirana,S.R., Subhash, N. and Jadeja, G.C.(2010).Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers. *Ind. Crops Prod*. **32**: 491–498.

Garcia, A.A.F., Benchimol, L.L., Barbosa, A.M.M., Geraldi,I.O., Souza, C.L.& Souza, A. Pd. (2004). Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. *Genet. Mol. Biol*. **27**: 579–588.

Govindaraj,M., Vetriventhan, M. & Srinivasan,M.(2015). Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. *Genet. Res. Int*. article ID 431487.

Grover, J.K. & Yadav, S.P. (2004). Pharmacological actions and potential uses of *Momordica charantia*: a review. J. Ethnopharmacol. **93**: 123–132.

Gupta, M., Sharma, S., Gautam, A.K. & Bhadauria, R.(2011). Momordica charantia Linn. (karela): nature's silent healer. *Int. J. Pharm. Sci. Rev. Res*. **11**: 32–37.

Hoque, S. & Rabbani,M.G. (2009). Assessment of genetic relationship among landraces of Bangladeshi ridge gourd (*Luffa acutangula* Roxb.) using RAPD markers. *J. Sci. Res*. **1**: 615–623.

Islam, M.R., Hossain, M.S., Bhuiyan,M.S.R., Hasan, G.N. & Syed,M.A.,.(2010). Multivariate analysis of bitter gourd (*Momordica charantia* L.). Middle East J. Sci. Res. 5, 86–90.

Islam, M.S., Mia, M.A.B., Das, M.R. & Hossain, T. (2014). Sex phenology of bitter gourd (*Momordica charantia* L.) landraces and its relation to yield potential and fruit quality. *Pak. J. Agric. Sci*. **51**: 649–656.

Kumar, N. & Khurana,S.M.P. (2016). Nutritive and antidiabetic benefits of *Momordica charantia* L. *Indo Am. J. Pharm. Sci*. **3**: 1512–1520.

Levi,A., Thomas, C. E., Newman,M., Reddy,O. K., Zhang, X. & Xu,Y. (2004).ISSR and AFLP Markers Differ among American Watermelon Cultivars with Limited Genetic Diversity, *J. Am. Soc. Hortic. Sci*. . **129(4)**: 553-558.

Lynch, M. & Walsh, B. (1998). Genetics and analysis of quantitative traits. Sinauer Associates, Inc., Sunderland.

Mishra,S., Behera, T.K. & Munshi,A.D. (2015) Induction and morphological characterization of hermaphrodite flowers in a gynoecious line of bitter gourd by silver nitrate, gibberellic acid and silver thiosulfate. *Int. J. Veg. Sci*. **21**: 204–211.

Pandey, P., Ansari, A.W., Kashyap, S.P., Bhardwaj,D.R., Tiwari, S.K. & Singh,B.(2019). Genetic diversity of Indian bitter gourd (*Momordica charantia*) by ISSR and morphological markers. *Indian J. Agric. Sci*. **89**: 2037–2042.

Pandey, S., Ansari, W.A., Mishra,V.K., Singh, A.K. & Singh, M. (2013) Genetic diversity in Indian cucumber based on microsatellite and morphological markers. *Biochem. Syst. Ecol*. **51**: 19–27.

Paris,H.S. (2002). Genetic control of irregular striping, a new phenotype in *Cucurbita pepo*. *Euphytica* **129**: 119–126.

Payel, D.E., Mala,P. & Sunita, S. (2015). Inter-genus variation analysis in few members of *Cucurbitaceae* based on ISSR markers. *Biotechnol. Equip*. **29**: 882–886.

Rathod, V., Narasegowda, N.C., Papanna, N. & Simon, L. (2008). Evaluation of genetic diversity and genome fingerprinting of bitter gourd genotypes (*Momordica charantia* L.) by morphological and RAPD markers. *Int. J. Plant Breed*. **2**: 79–84.

Rohlf, F.J. (2000). NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System Version 2.1. Exeter Publishing Setauket, New York.

Saleh, B. (2011). Efficiency of RAPD and ISSR markers in Assessing Genetic Variation in *Arthrocnemum macrostachyum* (Chenopodiaceae). *Braz. Arch. Biol. Technol*. **54**: 859–866.

Saxena, S., Singh, A., Archak, S., Behera, T.K., John, J.K., Meshram, S.U. and Gaikwad, A.B. (2015). Development of novel simple sequence repeat markers in bitter gourd (*Momordica charantia* L.) through enriched genomic libraries and their utilization in analysis of genetic diversity and cross-species transferability. *Appl. Biochem. Biotechnol*. **175**: 93–118.

Singh, A.K., Behera, T.K., Chandel, D., Sharma, P. & Singh NK (2015). Assessing genetic relationships among bitter gourd (*Momordica charantia* L.) accessions using inter- simple sequence repeat (ISSR) markers. *J. Hortic. Sci. Biotechnol*. **82**: 217–222.

Singh, B., Singh, A.K.& Kumar, S. (2013). Genetic divergence studies in bitter gourd (*Momordica charantia* L.). *Acad. J. Plant Sci*. **6**: 89–91.

Souframanien, J. & Gopalakrishna, T. (2004). A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. *Theor. Appl. Genet*. **109**: 1687–1693.

Thomas, T.D. (2008).The effect of in vivo and in vitro applications of ethrel and GA_3 on sex expression in bitter melon (*Momordica charantia* L.). *Euphytica* **164**: 317–323.

Verma, K.S., Haq, U.S., Kachhwaha, S. & Kothari, S.L. (2017). RAPD and ISSR marker assessment of genetic diversity in *Citrullus colocynthis* (L.) Schrad: a unique source of germplasm highly adapted to drought and high-temperature stress. *Biotech*, **7**: 288.

Verma, V.K., Behera, T.K., Munshi, A.D., Parida, S.K.& Mohapatra, T.(2007). Genetic diversity of ash gourd [*Benincasa hispida* (Thunb.) Cogn.] inbred lines based on RAPD and ISSR markers and their hybrid performance. *Sci. Hortic*. **113**: 231–237.

Yang, S.L. & Walters, T.W. (1992) Ethnobotany and the economic role of the Cucurbitaceae of China. *Econ. Bot*. **46**: 349–367.

Yeh, G.Y., Eisenberg, D.M., Kaptchuk, T.J. & Phillips, R.S. (2003). Systematic review of herbs and dietary supplements for glycemic control in diabetes. *Diabetes Care*. **26**: 1277–1294.

Zietkiewicz,E., Rafalski, A. & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*. **20**: 176–183.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.15

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Comparative Analysis of Nutritional Values, Bioactive Compounds and Antinutrients in Tartary and Common Buckwheat Leaves

Nidhi Joshi, Kumkum Giri, Aditi Biswas, Pawanesh Tamta and Kuldip C. Verma*

Abstract

Among the millets grown in the Himalayan region of India, common and tartary buckwheat are the most cultivated species. Due to its extensive nutritional and antioxidant characteristics, buckwheat is attracting much interest, especially in producing nutrient-rich healthy foods. The objective of this study was to investigate nutritional values (protein, lysine, arginine and tryptophan content), bioactive compounds (total phenolic and flavonoid contents, antioxidant and rutin content) and antinutrients (phytate, tannin and oxalate) in tartary and common buckwheat leaves. Till now, very little information is available on the phytochemical constituents of buckwheat leaves as compared to the seeds. Common buckwheat leaves contain significantly ($p < 0.05$) more protein (22.21 ± 0.23 gm/100 g), lysine (12.41 ± 0.41 gm/ 100 g protein), arginine (6.61 ± 0.29 gm/100 g protein) and tryptophan (3.58 ± 0.36 gm/100 g protein) content then tartary buckwheat. There were relatively minor differences in the contents of amino acids. The results showed that the phenol (1768.16 ± 0.78 mg GAE/100 g), flavonoid (678.31 ± 0.27 mg QE/100 g), total antioxidant activity (52.89 ± 0.77 mg/100 g), rutin (11.32 ± 0.10 gm/100 gm), phytate (2.84 ± 0.07 gm/100 gm), tannin (2.76 ± 0.08 gm/100 gm), and oxalate (4.27 ± 0.03 gm/100 gm) content of the tartary buckwheat samples were significantly ($p < 0.05$) higher as compared to that of the common buckwheat leaves. On the basis of this study, it can be concluded that tartary buckwheat leaves are superior as green vegetables compared to common buckwheat for preventative nutrition.

Keywords: Buckwheat, Phenol, Flavonoid, Lysine, Antinutrients, Rutin

Department of Biochemistry, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

***Author for correspondence:**

kc.verma@gbpuat-cbsh.ac.in

Received: 03/08/2023 **Revised:** 24/08/2023

Accepted: 24/08/2023

How to cite this article: Joshi, N., Giri, K., Biswas, A., Tamta, P., Verma, K.C. (2023). Comparative Analysis of Nutritional Values, Bioactive Compounds and Antinutrients in Tartary and Common Buckwheat Leaves. *Indian J. Plant Genetic Resources*. 36(3), 449-454.

DOI: 10.61949/0976-1926.2023.v36i03.15

Introduction

Buckwheat is an ancient worldwide accepted, nutrient dense and healthy crop of Polygonaceae family and is cultivated in diverse ecological regions mostly to produce grains for human sustenance. Buckwheat, in India, is grown chiefly in hilly areas of Jammu and Kashmir, Uttarakhand, Himachal Pradesh, Uttar Pradesh, Darjeeling, upper Assam region, Nilgiris, Sikkim and Tamil Nadu and Kerala hills (Phull and Gupta 2023). *Fagopyrum* is a small genus consisting of two cultivated species and over 25 wild buckwheat species, the majority of which are endemic to southwest China, which is regarded the origin, distribution, and diversity centre. Common buckwheat (*F. esculentum* Moench) and tartary buckwheat (*F. tataricum* Gaertn) are two species that are widely cultivated and spread all over the world. Both cultivated species have health benefits, are gluten-free, and have one of the most balanced amino acid composition of any cereal product (Luthar *et al*., 2021). Buckwheat is an annual or perennial herb that grows 0.5 to 2.5 m tall and has hollow, branching or unbranched reddish stems with white or pink blossoms. Plants grow rapidly, with little heart-shaped leaves and slender hollow stems (Ahmed *et al*., 2014). Buckwheat is an underutilized food crop that is gaining popularity,

especially in the development of nutrient-rich health foods due to its extensive nutritional, therapeutic, and antioxidant characteristics (Kumari & Chaudhary 2020). Buckwheat is an annual plant that is grown for both its seeds and its leaves. Buckwheat leaves and flowers are utilised in traditional medicine in India. Buckwheat leaves are used in the hills of Uttarakhand as a green leafy vegetable (hara saag).

Tea is made from the leaves of plants, which lowers cholesterol and glucose levels in the blood. The high amount of phenolic compounds and dietary fibre in buckwheat leaves contributes to these qualities. Buckwheat seeds are also recognised as an important dietary source due to their high nutritional value proteins and other bioactive compounds. Buckwheat seeds are processed into groats, flour, pasta, and bread (Dziadek *et al*., 2018). Buckwheat has grown in popularity as a health food in several countries in recent years, due to its capacity to grow in unfavourable climatic conditions and low cultivation cost. Buckwheat holds a lot of promise as a substitute staple crop because it meets all of the requirements present in commonly cultivated crops (Rana *et al*., 2012). Rutin, a valuable antioxidant found in abundance in buckwheat leaves, is used extensively in the cosmetic and pharmaceutical industries. It is a common functional component of buckwheat, having biological properties such as anti-hypertension activity, lowering cholesterol levels, reducing headaches and fatigue, and lowering body mass index, as well as anti-inflammatory, antithrombotic, and cytoprotective activities. It can also aid in the prevention of cancer and cardiac disease (Saraswat & Kumar, 2019**).** Most of the studies are focus on chemical composition of buckwheat seeds as well as health properties. There are little data concerning the study of other plant parts of buckwheat. Therefore, this study aimed to determine the nutritional composition, bioactive compounds, antioxidants and ant-nutrients in leaves between most cultivated buckwheat species in Uttarakhand.

Material and Methods

Collection and cultivation of buckwheat species

Seed samples of both the species (*F. esculentum* and *F. tataricum*) were collected from Magroli and Jageshwar regions of Uttarakhand. Seeds were dried and cleaned to remove all the foreign particles and damaged seeds. The dried and cleaned seed samples were packed in glass jars and stored at room temperature in dark condition. Seeds were grown at Norman E. Borlaug Crop Research Centre, Pantnagar in November 2022 and the plant leaves were used in the study.

Preparation of extract

Leaves were dried in natural condition [at ambient temperature (20–25°C) in a shaded, well-ventilated area]. Natural drying is the most popular method for preservation of herbs in various types of plants. The methanolic extract were prepared as per A.O.A.C., 1965. In this method, powdered samples (100 g) of buckwheat were wrapped in muslin cloth and placed in the thimble of the soxhlet apparatus. Methanol (80%) was used as solvents for extract preparation (0.5 mg/mL). Soxhlet was run at 50°C temperature for 8 hours. Solvents were separated using distillation unit, followed by further concentrating the extracts on water bath.

Estimation of total phenol content

The total phenolic content (TPC) was ascertained using the Folin-Ciocalteu method (Bray and Thorpe, 1954). A 0.5 mL (1 mg/10 mL) aliquot of methanol was taken in the test tubes. A volume of 3 mL of distilled water and 0.5 mL of 2.0 M Folin-Ciocalteu reagent were added. The tube was slowly filled with 2 mL of a 20% (w/v) Na_2CO_3 solution after 3 minutes of incubation. The absorbance at 650 nm was measured following 60 minutes of incubation at room temperature. Using a calibration curve for 98% gallic acid, the total phenol content was determined and expressed as mg/100 g of dry wt. sample.

Estimation of total flavonoid content

The $AlCl_3$ method was used to calculate the total flavonoid content (TFC) (Jagadish *et al*., 2009). The test tubes were filled with a 0.5 mL aliquot of the methanolic extract, and the volume was kept constant at 1-mL using methanol 0.3 mL of 5% $NaNO_2$ and 4 mL of dH_2O were used to prepare the blank. Then, 1.5 mL of a 2% $AlCl_3$ solution was added after 5 minutes. After adding 2 mL of 1 M NaOH to each tube, 10 mL of distilled water was then added. The mixture was vortexed 10 minutes after it had been incubated. Different quercetin concentrations (20, 40, 60, 80, and 100 g/mL) were used to make the standard curve, and the absorbance was measured at 367 nm. Total flavonoid content was expressed in 98% quercitin milligrams per 100 gm of dry wt. sample.

Estimation of total antioxidant capacity

The total antioxidant capacity was determined by Prieto *et al*., (1999) using a phosphomolybdenum-based spectrophotometric technique. Methanolic extract (0.5 mL) and 80% methanol were combined in a test tube, and then 3 mL of phosphophomolybdenum reagent was added. The test tube was then incubated for 90 minutes at 95°C in a water bath. At 37°C, samples were allowed to cool. The standard was ascorbic acid (1-mg/mL), and the absorbance was determined at 695 nm. In terms of ascorbic acid equivalent, the total antioxidant activity was measured in gram per 100 gram of dw sample.

Estimation of crude protein

The Kjeldahl method was used to determine crude protein (Lynch and Barbano, 1999). Digestion, distillation, and titration are the three main steps. Strong acids, such as

sulfuric acid, were used to digest the leaf samples, and bases were used for distillation and an indicator dye for titration. After that, nitrogen content was calculated using the formula below:

$$\%\text{Nitrogen} = \frac{\text{Titre value} \times 0.00014}{\text{Weight of the sample}} \times \frac{\text{Volume made up}}{\text{aliquot of distilled}} \times 100$$

Crude protein content (g/100 g of dry wt. sample) was calculated by multiplying nitrogen content by conversion factor (6.25).

Estimation of lysine content

An established given by Sadasivam and Manickam (1992) was used to ascertain the lysine content. Defatted leaf sample 100 mg was taken, 5 mL papain solution was added and incubated at 65°C whole night. At room temperature, it was centrifuged at 3000 rpm for 10 minutes. In a centrifuge tube, supernatant (1-mL) was combined with copper phosphate reagent (0.5 mL) and carbonate buffer (0.5 mL) followed by centrifugation. Supernatant (1-mL) was mixed with 0.1 mL of 2-chloro-3, 5-dinitrophyridine solution. After 2 hours 5 mL of 1.2 N HCl was added and in a separatory flask 5 mL of ethyl acetate was added in previously obtained, top layer was removed. At 390 nm, the absorbance was calculated against a reagent blank (papain). For lysine concentrations of 40 to 200 g/mL, the standard curve was created and lysine content measured as mg/100 g of protein through standard graph.

Estimation of arginine content

The Sakaguchi method was used to calculate the arginine content (Tomlinson and Viswanatha, 1974). Defatted buckwheat leaf powder (100 mg) were hydrolyzed at 120°C for 8 hours with 2 mL of HCL (6 N). Sodium hydroxide was used for neutralization, and distilled water was used to dilute it to 40 mL. α -naphthol (0.1%) and KOH (10%) both 1-mL were added to 0.5 mL of the neutralized mixture and thoroughly blended. Following the addition of a drop of urea (5%) and 2 mL of potassium hypobromite, the mixture was continuously shaken. The arginine solution was substituted for distilled water to prepared a reagent blank, which was then incubated for 20 minutes at room temperature. A standard curve was created using various 20 to 100 mg/mL of 99% pure arginine concentrations by taken absorbance at 520 nm. The arginine content was expressed as g/100 g of dry wt. sample.

Estimation of tryptophan content

Spectrophotometric method was used to determine the tryptophan content of leaf samples (Spies and Chambers, 1949). A conical flask containing defatted 50 mg leaf powder, 30 mg p-dimethyl amino-benzaldehyde, and 10 mL of H_2SO_4 (19N) was thoroughly mixed, and the mixture was then left at 37°C in the dark for 12 hours. After incubation, 0.1 mL of $NaNO_2$ (0.045%) was added, the mixture was centrifuged for 15 minutes at 5000 rpm. Tryptophan was used to draw the standard graph, absorbance was measured at 454 nm, and finally, the amount of tryptophan was expressed in mg/100 g of protein.

Estimation of rutin

The amount of rutin was calculated based on spectrophotometric method (AOAC, 1995). Extract was prepared by 100 mg of leaf powder using methanol: aceticacid: water (100:2:100 V/V) for 60 minutes at room temperature. The absorbance of the diluted sample was measured at 352 nm. Rutin content (%) in samples was calculated using standard curve of rutin.

Estimation of tannin content

The total tannin content was determined using the spectrophotometric method (Chandran and Indira, 2016). A volumetric flask containing 0.5 mL of methanolic extract, 0.5 mL of distilled water, 1-mL of Na_2CO_3, and 0.5 mL of Folin-Ciocalteu reagent (FCR) was properly mixed followed by incubation for 30 minutes at room temperature. The same procedures were used to create a tannic acid standard calibration curve for a range of concentrations (20–100 µg/mL). At 700 nm, the absorbance was calculated. A standard graph was used to calculate the tannin acid content (measured in mg of tannic acid equivalents per 100 g of dry wt. sample).

Estimation of phytic acid content

To calculate the amount of phytic acid, the spectrophotometric method described by Haug and Lantzsch, 1983. The 0.5 g of powdered leaf sample was extracted using 0.2 N, 25 mL HCl for 3 hours on a shaker and then filtered through No. 1 Whatman filter paper. Filtrate (0.5 mL) was taken from the mixture, added to 0.9 mL of distilled water, 1-mL of ferric ammonium sulphate, and 30 minutes of incubation was done in a boiling water bath. After mixing 1.5 mL of freshly made bipyridine solution and 1-mL of the supernatant in a blank tube, absorbance was measured at 519 nm. Utilising a standard sodium phytate graph, the amount of phytic acid in the sample was calculated and expressed as g of phytic acid per 100 g of dry wt. sample.

Estimation of oxalate content

The method was described by Baker, 1952 in a volumetric flask, 2 gm of powdered buckwheat leaf sample was digested for one hour with 10 mL of 6M HCl before being filtered through Whatman No. 1 filter paper. Using a concentrated NH_4OH solution, the pH of the filtrate was raised until a pale yellow color appeared in place of the salmon pink color. Following this, the insoluble oxalate was precipitated from the filtrate using 10 mL of $CaCl_2$ solution (5%) and centrifuged for 10 minutes at 2500 rpm. The precipitate obtained was redissolved in 10 mL of 20% H_2SO_4. The filtrate's volume was increased to 200 mL, and then it was heated till boiling. This filtrate was titrated for 30 seconds with a 125 mL aliquot of 0.05 M standard potassium permanganate solution, and the

volume of the standard solution used was recorded. The titre value was used to calculate the oxalate content in mg/100 gof dry wt. Sample.

Statistical Analysis

All the experiments were being performed three times and one-way analysis of variance (ANOVA) was done on a program developed by Computer, Mathematics and Statistics Department of Pantnagar University, to determine variations in the estimated phytoconstituents with respect to the species and with other biochemical parameters. All readings were presented as mean ± SD (standard deviation).

Results and Discussion

Total phenol content (TPC) in leaves of common buckwheat was 1477.47 ± 0.98 and 1768.16 ± 0.78 mg GAE/100 gm of dry wt. in tartary buckwheat leaves. The TFC of the common and tartary buckwheat leaves was 561.79 ± 0.65 and 678.31 ± 0.27 mgQE/100 gm of dry wt. sample respectively. Obtained results were in agreement with Jiang *et al*., 2007, who stated that tartary buckwheat seeds has been shown to contain a higher content of flavonoids in comparison with common buckwheat. It was also reported that tartary buckwheat exhibited a remarkably higher TPC and TFC compared to common buckwheat (Liu *et al*., 2019). Total antioxidant activity in common and tartary buckwheat was 41.63 ± 0.62 and 52.89 ± 0.77 mg/ 100 gm of dry wt. sample. It is reported that antioxidant capacity of the common buckwheat was significantly lower than that of tartary buckwheat (Tien *et al*., 2018). Rutin content was 8.75 ± 0.07 and 11.32 ± 0.10 g/100 gm of dry wt. in common and tartary buckwheat, respectively (Table 1). Tartary buckwheat seeds contained more rutin than common buckwheat seeds (Fabjan *et al*., 2003).

Tartary buckwheat grains possessed three to four times more antioxidative activity then common buckwheat grains, and their rutin content was more than ten times that of common buckwheat (Lee *et al*., 2016). Polyphenols contribution to antioxidative activity differs between common and tartary buckwheat. Not only rutin content was lower in common buckwheat than tartary buckwheat, but so were other polyphenol components (Morishita *et al*., 2007). Tartary buckwheat has higher concentrations (at least two fold) of 61 flavonoids and 94 non-flavonoid secondary metabolites than common buckwheat. It is proposed that tartary and common buckwheat grains are both rich in secondary metabolites that are beneficial to human health. Non-flavonoid metabolites, especially, may have contributed to tartary buckwheat's better health-promoting value as compared to common buckwheat. Plants of common and tartary buckwheat survived at high altitudes by gradually accumulating genes for secondary metabolite synthesis, which allowed the plants to survive and reproduce in the less favorable environment. The richness and diversity of tartary buckwheat compounds protect plants from UV radiation, pathogens, and grazing (Kreft *et al*., 2022).

High protein content was observed in the common buckwheat leaves 22.21 ± 0.23 gm/100 g dry wt. as in tartary buckwheat *i.e.* 19.27 ± 0.52 g/100 gm of dry wt. Sample (Table 2). Qin *et al*., 2010 reported that there were no significant differences in protein content between tartary buckwheat flour and common buckwheat flour on average. The protein content of the tartary buckwheat groats was higher than that of the common or tartary buckwheat groats (Tien *et al*., 2018). The lysine content was 12.41 ± 0.41 g min the leaves of common buckwheat, while 10.91 ± 0.36 g/100 gm in dry wt. of protein in tartary buckwheat leaves. More arginine content was observed in the leaf of common buckwheat *i.e.* 6.61 ± 0.29 g and the slightly lower was found in the tartary buckwheat *i.e.,* 5.64 ± 0.32 g/100 gm dry wt.

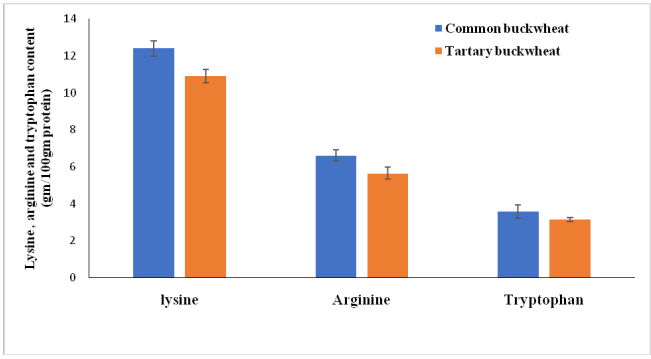


Figure 1: Lysine, arginine and tryptophan content (g/100 gm protein) in common and tartary buckwheat

Table 1: Phenol, flavanoid, antioxidant and rutin content of common and tartary buckwheat leaves

Parameters / *Species*	*Total phenol content (mg GAE/100 g of dry wt.)*	*Total flavonoid content (mg QE/100g of dry wt.)*	*Antioxidant (mg/ 100g of dry wt.)*	*Rutin (g/100g of dry wt.)*
Common Buckwheat	1477.47 ± 0.98	561.79 ± 0.65	41.63 ± 0.62	8.75 ± 0.07
Tartary Buckwheat	1768.16 ± 0.78	678.31 ± 0.27	52.89 ± 0.77	11.32 ± 0.10
CD at 1%	40.42295	32.14022	1.930120	.5736279
CD at 5%	28.84563	22.93511	1.377325	.4093383
SEM	9.363048	7.444543	.4470680	.1328677

Table 2: Protein, lysine, arginine tryptophan, phytate, tannin and oxalate content of common and tartary buckwheat leaves

Parameters / Species	Protein	Lysine	Arginine	Tryptophan	Phytic acid	Tannin	Oxalate
Common Buckwheat	22.21 ± 0.23	12.41 ± 0.41	6.61 ± 0.29	3.58 ± 0.36	2.23 ± 0.19	2.11 ± 0.07	4.40 ± 0.20
Tartary Buckwheat	19.27 ± 0.52	10.91 ± 0.36	5.64 ± 0.32	3.14 ± 0.12	2.84 ± 0.07	2.76 ± 0.08	4.27 ± 0.03
CD at 1%	.8936064	.7803922	.4605601	.4047715	.6021335	.1659603	.2958711
CD at 5%	.6376734	.5568843	.3286535	.2888431	.4296797	.1184285	.2111322
SEM	.2069834	.1807599	.1066782	.09375602	.1394704	.03844089	.06853174

*Unit of all the parameters in the above table is g/100 gm dry wt. of sample

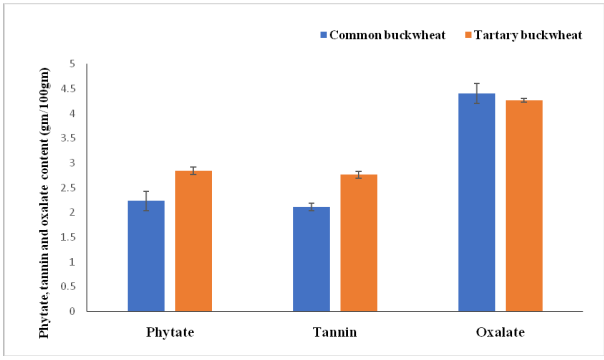


Figure 2: Phytate, tannin and oxalate content in common and tartary buckwheat

of protein. Tryptophan content in the leaves of common buckwheat was 3.58 ± 0.36 g, while in tartary buckwheat 3.14 ± 0.12 g/100 gm dry wt. of protein (Table 2, Figure 1). Amino acid profile is comparatively similar in both the buckwheat species (Bonafaccia *et al*., 2003)

Phytic acid content was 2.23 ± 0.19 and 2.84 ± 0.07 g/100 gm dry wt. sample in common and tartary buckwheat (Table 2, Figure 2).It is reported that tartary buckwheat having considerable higher content of phytic acid (Bonafaccia and Fabjan 2003). The tannin content in common and tartary buckwheat was 2.11 ± 0.07 and 2.76 ± 0.08 g/100 gm dry wt. Similar findings were also reported by Gadžo *et al*., 2010. The oxalate content in common and tartary buckwheat samples was 4.40 ± 0.20 and 4.27 ± 0.03 gm/100 gm of dry wt. Buckwheat genotypes showed a significant ($p \leq 0.05$) variation in oxalate content present in seeds (Dogra and Awasthi 2015).

Conclusion

There were significant (≤5%) phytochemical differences among cultivated buckwheat species in Himalayan regions. It may be due to the geographical locations, environmental conditions, harvesting time and genetic factors. The leaves of tartary buckwheat had significantly higher TPC, TFC, rutin, and antioxidant capacity than those of the common buckwheat. Tartary buckwheat was found to be a good source of nutrients and functional components and could be used for food processing. Higher amount of rutin content in tartary buckwheat will pave the way to fulfil the demand of rutin in cosmetic and pharmaceutical industries. Common buckwheat is also an important food, as it contains proteins with high biological value and balanced amino acids (lysine, arginine and tryptophan). This study will be helpful in improving various traditional buckwheat food products as per nutritional point of view. High value of rutin in tartary buckwheat will also boost the food, cosmetic and pharmaceutical industries.

Funding

This work was supported by Uttarakhand State Council for Science and Technology [Grant numbers:UCS&T/R&D-06/21-22/20248/1, Dated: 04.01.2022].

References

Ahmed A, N Khalid, A Ahmad, NA Abbasi, MSZ Latif, and MA Randhawa, (2014) Phytochemicals and biofunctional properties of buckwheat: a review. *J Agric Sci*. **152(3)**: 349-369.

AOAC (1995) Official methods of Analysis, Washington: Association of Analytical Chemist, 29

Association of Official Analytical Chemists (A.O.A.C.) (1965). Methods of Analysis. 11th ed. Washington, D.C.

Baker CJL (1952) The determination of oxalates in fresh plant material. *Analyst*. **77(916)**: 340-344.

Bonafaccia G, and N Fabjan (2003) Nutritional comparison of tartary buckwheat with common buckwheat and minor cereals. *Zb. Bioteh. Fak. Univ. Ljublj. Kmet*. **81(2)**: 349-355.

Bonafaccia G, M Marocchini, and I Kreft (2003) Composition and technological properties of the flour and bran from Common and Tartary buckwheat. *Food Chem*. **80(1)**: 9–15.

Bray HG. and WV Thorpe (1954) Analysis of phenolic compounds of interest of metabolism. In: 'Glick D (Eds.) Methods of biochemical analysis.' Interscience Publishers, Hoboken, New Jersey, pp 27-52

Chandran KCI, and G Indira (2016) Wound healing activity of ethanolic extract of roots of *Morindapubescens* JE Smith. *J. pharmacogn. phytochem*. **5(3)**: 43-46.

Dogra D, & CP Awasthi (2015) Comparative nutritional evaluation of common buckwheat genotypes with major cereal and pseudocereals crops. *Agric. Sci. Dig*. **35(1)**: 36-40.

Dziadek K, A Kopeć, E Piątkowska, T Leszczyńska, E Pisulewska, R Witkowicz, and R Francik (2018) Identification of polyphenolic compounds and determination of antioxidant activity in extracts and infusions of buckwheat leaves. *Eur. Food Res.*

Technol. **244**: 333-343.

Fabjan N, J Rode, IJ Košir, Z Wang, Z Zhang, & I Kreft (2003) Tartary buckwheat (*Fagopyrum tataricum* Gaertn) as a source of dietary rutin and quercitrin. *J. Agric. Food Chem*. **51(22)**: 6452-6455.

Gadžo D, M Djikić, T Gavrić, and JP Štrekel (2010) Comparison of tannin concentration in young plants of common and tartary buckwheat. *Acta Agric. Slov*. **95**: 75-78.

Haug W, and HJ Latzsch (1983) Sensitive method for the rapid determination of phytate in cereal and cereal products. *J. Sci. Food Agric*. **34(12)**: 1423-1426.

Jagadish LK, VV Krishnan, R Shenbhagaraman, and V Kaviarasan (2009) Comparative study on antioxidant, anticancer and antimicrobial property of *Agaricus bisporus*(JE lange) Imbach before and after boiling.*Afr. J. Biotechnol*. **8(4)**: 654-661.

Jiang P, F Burczynski, C Campbell, G Pierce, JA Austria and CJ Briggs (2007) Rutin and flavonoid contents in three Nutritional profile of buckwheat 365 buckwheat species *Fagopyrum esculentum*, *F. tataricum*, and *F. homotropicum* and their protective effects against lipid peroxidation. *Food Res. Int*. **40**: 356–364.

Kreft I, A Vollmannová, J Lidiková, J Musilová, M Germ, A Golob, and Z Luthar (2022) Molecular shield for protection of buckwheat plants from UV-B radiation. *Molecules*. **27(17)**: 5577.

Kumari A, and HK Chaudhary (2020) Nutraceutical crop buckwheat: a concealed wealth in the lap of Himalayas. *Crit. Rev. Biotechnol* . **40(4)**: 539-554.

Lee LS, EJ Choi, CH Kim, JM Sung, YB Kim, DH Seo, ... and JD Park (2016) Contribution of flavonoids to the antioxidant properties of common and tartary buckwheat. *J. Cereal Sci*. **68:** 181-186.

Liu Y, C Cai, Y Yao, and B Xu (2019) Alteration of phenolic profiles and antioxidant capacities of common buckwheat and tartary buckwheat produced in China upon thermal processing. *J. Sci. Food Agric*. **99(12)**: 5565-5576.

Luthar Z, P Fabjan, and K Mlinarič (2021) Biotechnological methods for buckwheat breeding. *Plants*. **10(8)**:1-22.

Lynch JM, and DM Barbano (1999) Kjeldahl nitrogen analysis as a reference method for protein determination in dairy products. *J. AOAC Int*. **82(6)**: 1389-1398.

Morishita T, H Yamaguchi, and K Degi (2007) The contribution of polyphenols to antioxidative activity in common buckwheat and tartary buckwheat grain. *Plant Prod. Sci*. **10(1)**: 99-104.

Phull T, & M Gupta (2023) Extraction process optimization for antioxidants, nutritional, physicochemical and functional properties in Fagopyrum esculentum Moench leaves of Himalayan region. *Food and Humanity*. **1**: 696-709

Prieto P, M Pineda, and M Aguilar (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. *Biochem*. **269(2)**: 337-341.

Qin P, Q Wang, F Shan, Z Hou, and G Ren (2010) Nutritional composition and flavonoids content of flour from different buckwheat cultivars. *Int. J. Food Sci. Technol*. **45(5):** 951-958.

Rana JC, RC Chauhan, TR Sharma, and N Gupta (2012) Analyzing problems and prospects of buckwheat cultivation in India. *Eur. J. Plant Sci. Biotechnol*. **6(2)**: 50-56.

Sadasivam S, and M Maickam (1992). Biochemical methods for agricultural sciences. Wiley Eastern limited, New Delhi. pp 45-46.

Saraswat R, and M Kumar (2019) Plant regeneration in buckwheat (*Fagopyrum esculentum* Moench.) via somatic embryogenesis and induction of meristemoids in abnormal embryos. *Plant tissue cult. Biotechnol*. **29(1)**: 33-47.

Spies JR, and DC Chambers (1949) Chemical determination of tryptophan in protein. *Anal. Chem*. **21**: 1349-1252.

Tien NNT, LND Trinh, N Inoue, N Morita, and PV Hung (2018) Nutritional composition, bioactive compounds, and diabetic enzyme inhibition capacity of three varieties of buckwheat in Japan. *Cereal Chem*. **95(5)**: 615-624.

Tomlinson G, and T Viswanatha (1974) Determination of the arginine content of proteins by the Sakaguchi procedure. *Anal. Biochem*. **60(1)**: 15-24.

Verma K C (2018) Biochemical Constituents of Buckwheat (*Fagopyrum esculentum* Moench) collected from different geographical regions of Himachal Pradesh. Mol. Biol. Rep. **45(6)**: 2681–2687.

Indian J. Plant Genet. Resour. 36(3): 455-513 (2023)

Plant Germplasm Registration Notice*

The Plant Germplasm Registration Committee (PGRC) of ICAR held its XXXXV[th] meeting on September 21[st], 2021 in virtual mode. 124 proposals were received and examined at ICAR-NBPGR out of which 71 proposals complete in all respects and reviewed by experts were considered for registration. 64 proposals with unique/novel features belonging to 30 species were finally recommended for registration. The information on registered germplasm is published with the purpose to disseminate the information to respective crop breeders for utilization of these genetic stocks in their crop improvement programmes.

1. Wazuhophek (IC639795; INGR21112), a rice (*Oryza sativa* L.) germplasm with tolerance to sheath blight and low soil P tolerance

V Prakasam[1*], Jyothi Badri[1], RM Sundaram[1], C Priyanka[1], GS Laha[1], MS Prasad[1], VP Bhadana[2], Ravindra Kale[1], Mahadeva Swamy HK[1], M Anila[1], Anantha MS[1] and LV Subba Rao[1]

[1]*ICAR-Indian Institute of Rice Research, Hyderabad-500030, Telangana, India*

[2]*ICAR-Indian Institute of Agricultural Biotechnology, Ranchi-834010, Jharkhand, India*

[*]*Email: vprakasam.iari@gmail.com*

Sheath blight (ShB) of rice is one of the major devastating diseases and responsible for 70% of yield losses and quality degradation. It is caused by necrotrophic soil born pathogen *Rhizoctonia solani* AG-1 IA which affects sheath, leaf and panicle. It produces water soaked brown color oval shaped lesions on sheath, irregular lesion on leaf and produces sclerotia during unfavorable conditions and maintains dormant period in soil which can act as a primary source of inoculum.

Screening was done by inoculation of cultured *R. solani* typha bits at maximum tillering stage (Bhaktavatsalam *et al.,*1978) and disease scoring was given based on relative lesion height (RLH) as per (0-9) Standard Evaluation System (SES, IRRI, 2014). Among the entries screened, 'Wazuhophek' a North East Indian landrace was identified as highly tolerant (Table 1) through repeated artificial screening in field condition for the past 6 seasons *viz.*, *Kharif*-2012, *Rabi*-2013, *Kharif*-2013, *Rabi*-2014 and *Kharif*-2014 (Dey *et al*., 2016; Dey *et al*., 2020).

To know the tolerance mechanism in Wazuhophek, expression of PR genes, defense enzymes and histo-pathological studies were conducted. The expression transcript s of defense related genes *viz.,* PR-1, PR-2, PR-3, PR-4, PR-5, PR-9, PR-10, PR-13, CHS, LOX, PAL and PPO were studied by using quantitative Real-time PCR (qRT PCR). The expression levels of PR-1, PR-3, PR-9 and PR-10 genes were 56.14, 95.85, 31.48, and 66.1% higher folds in Wazuhophek than IR50 at 72 hours after inoculation with *R. solani* (Roy *et al*., 2018).

Histopathological studies were conducted between two cultivars *i.e.,* Wazuhophek as tolerant and IR-50 as susceptible by infecting with *R. solani*. Observations were recorded at 24 hours post inoculation (hpi), 48 and 72 hpi. Rate of hyphae branching, density of mycelium was high and most of the area was occupied by infection cushions in susceptible IR-50 when compared to tolerant Wazuhophek (Roy, 2018).

In addition to sheath blight tolerance, Wazuhophek also showed tolerance to low soil P and interestingly we found that it is completely devoid of *Pup1* gene. It performed equally with the tolerant checks Kasalath and Swarna which consists of gene specific *pup-1* gene (Swami *et al.,* 2019). 98 germplasm lines including wazuhophek were screened for low soil P tolerance in the low soil P plot (available p <2 Kg ha^{-1}) and with normal soil P condition (available p > 20 Kg ha^{-1}) at ICAR- IIRR during *Kharif*-2014 along with the tolerant checks Kasalath and Swarna and susceptible checks Improved Samba Mahsuri (ISM), MTU 1010, and IR-64. A total of fifteen parameters *viz*., days to 50% flowering (DFF), plant height (PH), number of productive tillers per plant (NPT, nos.), flag leaf length (FFL), flag leaf width (FLW), panicle length (PL), shoot length (SL), root length (RL), root volume (RV), dry shoot weight (DSW), dry root weight (DRW), root to shoot ratio (RSR), grain yield per plant (GY), thousand grain weight (TGW) and biomass (BM) were recorded.

Stress indices parameters such as STI (stress tolerance index), YSI (yield stability index), YI (yield index) showed high value for Wazuhophek, revealing its tolerance ability to low soil P while other non *Pup1* genotypes got very low values

[*]*Edited by: Anju Mahendru Singh and Anjali Kak, Division of Germplasm Conservation, ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012, India*

Acknowledgement is due to Mr. Arup Das, YP for providing technical assistance in compilation.

Table 1: Disease reaction among rice germplasm under artificial inoculation during 2012 to 2020

S.No	*K-2012 (F)*	*R-2013 GH)*	*K-2013 (F)*	*R-2014 (GH)*	*K-2014 (F)*	*K-2017 (F)*	*K-2018 (F)*	*K-2019 (F)*	*K-2020 (F)*
Tetep (TC)	3	3	3	3	3	3	5	5	3
Wazuhophek	3	3	3	3	3	3	3	5	3
IR-50 (SC)	9	9	9	9	9	9	9	9	9
Sapetmaso	5	3	5	7	7	NT	NT	NT	NT
9 (B)	5	5	5	7	7	NT	NT	NT	NT
16 (B)	5	5	5	7	7	NT	NT	NT	NT
61 (B)	5	3	5	5	5	NT	NT	NT	NT
APMS 6B	3	5	5	5	7	NT	NT	NT	NT
495	3	5	5	7	5	NT	NT	NT	NT
APMS-6B	3	5	5	5	7	NT	NT	NT	NT
MR-1523	5	5	5	7	9	NT	NT	NT	NT
Meghalaya Lefara	5	3	3	5	7	NT	NT	NT	NT

TC-Tolerant check; SC-Susceptible check; NT-Not tested; F-Field; GH-Glass House

Table 2: Genotypic status of eighteen low soil P tolerant genotypes for *Pup 1* locus, based on *Pup 1* specific markers

S. No	Genotypes	Status for Pup 1 specific marker loci				Percent yield reduction under low P condition
		K 46-1	K46-K1	K 46-2	K 52	
1	Nagaram Mahripid	N	N	N	K	23.4
2	Meghalaya R ba Laispah	N	K	K	K	45.0
3	Megalaya Lakang	K	K	K	K	46.1
4	Nungshangphou	K	N	K	K	46.8
5	Kueashu	K	N	K	N	58.2
6	Deserkangbu	K	N	K	K	61.6
7	Wazuhophek	N	N	N	N	62.1
8	Churhchandpur	K	K	K	K	63.2
9	Sumi special	K	K	K	K	63.8
10	Phougak	N	N	K	N	64.4
11	Vishku	K	N	N	N	66.3
12	Chinapati	K	K	K	K	66.5
13	Moirangphou Angovba	N	K	K	K	69.0
14	Zunhiboto	K	K	K	K	69.2
15	Ayamaomaha	N	N	K	K	69.4
16	Yun Yokan Steo	N	N	K	K	70.7
17	Priya	K	N	K	N	70.8
18	China Ching	K	K	K	K	70.9
19	Swarna	K	K	K	K	66.5

'K', Kasalath specific allele for the marker locus; 'N', Nipponbare specific allele for the marker locus [a]Low soil P tolerant check variety ; [b]Low soil P tolerant germplasm line which is completely devoid of Pup 1 locus

and fall into highly sensitive category. Stress susceptibility index of Wazuhophek was less than zero indicating its tolerance while SSI was >1 for other non-*Pup1* genotypes and were highly sensitive to low soil P. Marker assisted

characterization of germplasm lines for *Pup1* locus based on *Pup1* specific markers revealed that full set of sequences associated with *Pup1* are present in the tolerant checks (Kasalath and Swarna) respect to all the four *Pup1*- specific markers and absent in the sensitive checks (Improved Samba Mahsuri, IR-64 and MTU 1010). The Wazuhophek possessed 'N' allele (i.e., non-tolerant allele) with respect to all the four *Pup1* specific marker loci but phenotypically tolerant to low soil P (Swami *et al.,* 2019, Table 2). Thus, Wazuhophek possess different mechanisms for low P tolerance and hence could serve as a novel source for low soil P tolerance and could help in diversification breeding programs aimed for low soil P tolerance.

References

Bhaktavatsalam G, Satyanarayana K, Reddy APK, John VT (1978). Evaluation of sheath blight resistance in rice. *Int Rice Res Newsl,* **3:** 9–10.

Chin JH, Gamuyao R, Dalid C, Bustamam M, Prasetiyono J, Moeljopawiro S, Wissuwa M, Heuer S (2011) Developing rice with high yield under phosphorus deficiency: Pup1sequence to application. *Plant Physiol,* **156(3):** 1202–1216

Gamuyao R, Chin HJ, Tanaka JP, Pesaresi P, Catausan S, Chery Dalid, Loedin SI, Mendoza TME, Wissuwa M, Heuer S (2012) The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. *Nature,* **88:**535–541

IRRI (2004). Standard evaluation system for rice. International Rice Research Institute, Los Banos, Manila.

Kale RR, Anila M, Swamy HKM, Bhadana VP, Durga Rani CV, Senguttuvel P, Subrahmanyam D, Anatha MS and Sundaram RM (2019). Screening of the recombinant inbred line mapping population of rice derived from cross Wazuhophek x Improved Samba Mahsuri for low soil P tolerance. *The J. Res. PJTSAU* **47(1):**11-16.

Puli Sasanka Roy, Prakasam V, Kotasthane A S, Sundaram R M, Laha G S, Srinivas Prasad M(2018). P32: In-planta expression pf pathogenesis related genes in rice cultivars in response to sheath blight caused by *Rhizoctonia solani* Kuhn. "Special national symposium on extension plant pathology during September 25-26, IGKV, Raipur-Indian Phytopathological Society, New Delhi, P 34

Susmita Dey, Badri J, Prakasam V, Bhadana VP, Eswari KB, Laha GS, Priyanka C, Aku R and Ram T. (2016). Identification and agro-morphological characterization of rice genotypes resistant to sheath blight. *Australas. Plant Pathol.,* **45:**145-153.

Susmita Dey, Jyothi Badri, Eswari KB and Prakasam V (2020) Diversity analysis for yield traits and sheath blight resistance in rice genotypes. *Electron. J. Plant Breed,* **11(1):** 66-64.

Wissuwa M, Yano M, Ae N (1998) Mapping of QTLs for phosphorus deficiency tolerance in rice (*Oryza sativa* L.). *Theor Appl Genet.* **97:**777–783.

2. Kataribhog (IC640647; INGR21113), a Non-Basmati Aromatic rice (*Oryza sativa* L.) germplasm with low glycemic index (45.72%)

Somnath Mandal[1*], Bidhan Roy[2] and Prudveesh Kantamraju[1]

Uttar Banga Krishi Viswavidyalaya, Coochbehar-736165, West Bengal, India

[*]Email: smandal8183@gmail.com

Glycemic index (GI) helps in estimation of blood glucose response. According to Choi *et al.*, 2012, GI for foods has three categories mentioned as low (55 or less), medium (56-69) and high (70 or more). Frequent intake of rice with high GI is increasingly associated with elevated risk of type II diabetes, obesity, coronary heart disease and other chronic conditions. Northern part of West Bengal is famous for growing popular local indigenous rice landraces known as folk rice as well as many traditional short and medium grain aromatic rice genotypes. These genotypes collected from different parts of Sub-Himalayan region of West Bengal and adjoining states and are being maintained in the rice repository of Uttar Banga Krishi Viswavidyalaya. We have screened those collections of indigenous aromatic rice germplasm for their nutritional attributes and identified an aromatic rice cultivar named 'Kataribhog' with *in-vitro* low glycemic index (GI) value of around 45.72%, which is even lower than the GI of recommended rice varieties for diabetic patients. Kataribhog is photo-period sensitive, with long duration, tall crop, possessing slender type grain, low yield potential (1.5–2.0 t/ha) and having total soluble sugar of 64.77%, reducing sugar content of 1.59%, non-reducing sugar content of 0.77% starch content of 54.57%, amylose content 20.43%, amylopectin content: 34.13%, resistant starch content 2.25%, protein content 6.43% and antioxidant activity (IC_{50}) with 1583.68 μgm/ml (1).

GI of powdered Kataribhog was estimated by using *in vitro* method following Kumar *et al.,* 2018. The absorbance was measured at 510 nm. Maltose (200 mg) was used as standard carbohydrate. Average values were used to plot curves followed by computing the area under the curve (AUC). The Hydrolysis index (HI) for each rice variety was calculated by dividing AUC of sample by that of maltose and expressed in percentage. The predicted glycemic index was calculated using the following formula (PGI) = 39.71 + (0.549 × HI). Among the screened genotypes most of the genotypes showed an average GI value of more than 60% glycemic index. The glycemic index (GI) of the Kataribhog was found to be 45.72% which falls under low glycemic index according to Choi *et al.*, 2012 as 55 or less as low GI foods. GI of Pusa Basmati 1121 has been reported as 58.41%.

References

Choi Y, Giovannucci E, Lee JE (2012). Glycaemic index and glycaemic load in relation to risk of diabetes-related cancers: a meta-analysis. *British J.Nutri.* **108(11):** 1934-1947.

Kumar A, Sahoo U, Baisakha B, Okpani OA, Ngangkham U, Parameswaran C, Sharma SG (2018) Resistant starch could be decisive in determining the glycemic index of rice cultivars. *J. Cereal Sci.* **79:** 348-353.

3. CRR747-12-3-B (IET26337) (IC640651; INGR21114), a rice (*Oryza sativa* L.*)* germplasm with drought tolerant and resistant to blast disease

NP Mandal*, Somnath Roy and Amrita Banerjee

Central Rainfed Upland Rice Research Station, ICAR-NRRI, Hazaribag-825301, Jharkhand, India

**Email: npmandal@hotmail.com*

The breeding line CRR 747-12-3-B (IET 26337) was derived from the cross Vandana*4/ C101A51//IR84984-83-15-862-B at CRURRS (ICAR-NRRI), Hazaribag following backcross breeding method. This line was evaluated as IET 26337 under direct seeded early group (E-DS) AICRIP trials during 2016 to 2018. It recorded superior yield performance under both severe and moderate drought stress situations with good early vigour, strong culm and good tillering ability. CRR 747-12-3-B is highly tolerant to reproductive stage drought and outperformed all the checks varieties such as Sahbhagi Dhan, Vandana, and Anjali across the locations under severe drought stress conditions by registering yield advantage of 51–102 and 15–292% during 2016 and 2018, respectively. Molecular marker assisted screening confirmed

Table 1: DUS characteristics of rice elite line 'CRR747-12-3-B

Characteristics	*CRR 747-12-3-B*	*Characteristics*	*CRR 747-12-3-B*
Basal Leaf sheath colour	Green	Flag leaf: attitude of blade	Horizontal
Leaf: anthocyanin colouration	Absent	Panicle: length of main axis	Medium (22.3cm)
Leaf sheath: anthocyanin colouration	Absent	Panicle: curvature of main axis	Semi-straight
Leaf: pubescence of blade surface	Weak	Panicle: no. per plant	Medium (15)
Leaf: auricles	Present	Spikelet: colour of tip of lemma	Yellowish
Leaf: anthocyanin colouration of auricles	Colourless	Lemma and palea: colour	Gold and gold furrows on straw
Leaf: collar	Present	Panicle: awns	Absent
Leaf: Anthocyanin colouration of collar	Absent	Panicle: attitude of branches	Erect - semi-erect
Leaf: shape of ligule	Split	Panicle: exertion	Mostly exerted
Leaf: colour of ligule	White	Decorticated grain: length	6.26 mm
Leaf: length of blade	Medium (30.9 cm)	Decorticated grain: width	2.04 mm
Leaf: width of blade	Medium (1.0 cm)	Decorticated grain: shape	Long slender
Culm strength	Strong (no lodging)	Decorticated grain: colour	White
Culm angle	Intermediate	Decorticated grain: aroma	Absent
Time of heading (50% plant with panicles)	Very early (67 days)	Milling recovery	64.90%
Flag leaf: attitude of blade	Horizontal	Head rice recovery	56.10%
Spikelet: density of pubescence of lemma	Weak	Apparent Amylose content	23.30%
Lemma: anthocyanin coloration of area below apex	Absent	Gel consistency	Soft
Lemma: anthocyanin coloration of apex	Absent	Maturity duration (days)	95
Spikelet: colour of stigma	Light green	Yield (t/ha)	2.3-4.0 (normal); 1.1-2.6 (under stress)
Stem: length	Short (97 cm)	Drought score (in SES scale)	3
Stem: anthocyanin colouration of nodes	Absent	Blast score (in SES scale)	4.1-5.4
Stem: intensity of anthocyanin colouration of internodes	Absent		

that CRR 747-12-3-B possesses three DTY QTLs for grain yield under reproductive stage drought stress (*qDTY*2.3, *qDTY*3.2 and *qDTY*12.1), *Phosphorus starvation tolerance 1* (*PSTOL1*) gene for tolerance to low-Phosphorus, and blast resistance gene *Pi-2*. This line is moderately resistance to rice leaf blast (scored 4.1 - 5.4 in 0-9 scale) based on multi-location screening in NSN1 and NSN2 under AICRIP and has good grain quality (Table 1). This new line can be further exploited to develop multiple tolerant high yielding rice varieties.

4. NWGR-13017 (IC637523; INGR21115), a rice (*Oryza sativa* L.) germplasm with resistance to leaf folder

MB Parmar, SS Thorat*, RK Gangwar, SG Patel, DB Prajapati, DJ Kacha, KS Prajapati and AM Maheta (Late)
Main Rice Research Station, Anand Agricultural University, Nawagam- 387540, Gujarat, India
**Email: sanjuthorat2@gmail.com*

The rice genotype NWGR-13017 was developed through a cross between SK-20 x IET- 19297 followed by pedigree method of selection at MRRS, AAU, Nawagam. The Geographical Coordinates Latitude and Longitude (22°47'55"N and 72°34'45"E) of MRRS, AAU, Nawagam, Ta and Dist. Kheda, Gujarat. The genotype was tested in state trials during *Kharif,* 2015 as Preliminary Evaluation Trial (PET) and during *Kharif* 2016 as Small Scale Varietal Trial-Late (SSVT-L) at different location of Gujarat state. In addition to this the genotype NWGR-13017 was tested under AICRIP in the multilocation leaf folder screening trials (LFST) during *Kharif,* 2016 and 2017.

Morpho-agronomic characteristics

The progeny of genotype NWGR-13017 was selected from the SK-20 x IET-19297 and cross was made in the year 2006 and F8 Bulk (1-1-1-2-2-2-1) in the year 2013. The donor parent SK-20 was selected from land race of Sukhwel which is early and tall. Another parent, IET- 19297 (DJP-1998-11-1-1-1) was developed by cross between TN-1 x INDCR-1940. The characters of genotype IET 19297 is mid early and long slender grain type. Ultimately, the resistance characters of NWGR 13017 were incorporated from different land races such as SK- 20, Dg Wg, Tsai Yuan Chung and INDCR-1940. Moreover, DNA fingerprinting profile of rice genotype NWGR 13017 was done by AFLP marker at Department of Agricultural Biotechnology, Anand Agricultural University, Anand, Gujarat.

The genotype NWGR-13017 came under late maturity group *i.e.,* seed to seed maturity is 138.5 to 139.5 days. It took 108.5 to 109 days for fifty per cent flowering, plant height between 123.0 to 135.5 cm, panicle length ranged from 26.1 to 27.4 cm, numbers of productive tillers 8.5 to 10.5 per plant, medium slender grain type (length 9.52 to 9.83 and breadth 1.79 to 1.90 mm), 1000 grain weight near about 21.85 to 22.96 g and average grain yield ranges between 3684 to 5228 kg/ha (Table 1).

Associate Characteristics

The field experiments were conducted during *kharif,* 2016 and 2017 for the screening of rice genotypes along with national susceptible check Taichung Native 1 (TN-1) and resistant check (W-1263) against leaf folder. These genotypes were screened with the two rows of 10 hills each genotype and nine rows of test genotype alternating with one row of susceptible check TN-1 with two replications against leaf folder at Main Rice Research Station, Anand Agricultural University, Nawagam as well as different AICRIP centers across the India during 2016 and 2017, respectively (Table 2). The screening was carried out by standard evaluation system (SES) for leaf folder (Anonymous, 2013). At 25 days after transplanting (DAT), the genotypes were covered with nylon net and leaf folder adults were released inside the net collecting from the neighboring fields. Adults were released at 40 and 60 DAT @ 100 adults in each release. Cotton dipped in 20% honey solution was placed inside the net to provide food for adults. Adults were allowed to remain in the net for a week, then the net was removed and observations were recorded at the time of peak infestation on selected ten randomly plants in each genotype. At each observation, total number of leaves and leaf folder damaged leaves were

Table 1: Morphological traits and grain yield of NWGR-13017 (Mean ranges of 2015 and 2016)

Characters	*Mean*	*Characters*	*Mean*
DFF (days)	109.0 (108.5 - 109.0)	Grain breadth (mm)	1.85 (1.79 - 1.90)
Days to Maturity	139.0 (138.5 - 139.5)	1000 grain wt. (g)	22.41 (21.85 - 22.96)
Plant height (cm)	129.0 (123.0 - 135.5)	Grain Yield (kg/ha)	4456 (3684 - 5228)
Panicle length (cm)	27.0 (26.1 - 27.4)	Grain type	Medium Slender
No. of productive tillers/plant	10.0 (8.5 - 10.5)	Flag leaf attitude of blade	Semi erect
Grain length (mm)	9.18 (9.52 - 9.83)	Length/Width of leaf blade	Short/Medium

Table 2: Performance of NWGR-13017 against leaf folder at different location across the India under LFST trial of AICRIP

Year	Name of genotypes	Per cent damaged leaves of leaf folder at different locations					Mean
		PTB (L1)	KUL (L2)	LDN (L3)	NWG (L4)	MSD (L5)	
Kharif, 2016	NWGR-13017	93.20	26.70	45.90	9.10	13.00	37.58
	W 1263 (RC)	92.50	12.80	26.10	8.90	1.00	28.26
	TN 1 (SC)	100.00	36.50	54.50	15.90	27.20	46.82

Year	Name of genotypes	Per cent damaged leaves of leaf folder at different locations							Mean
		PTB (L1)	KUL (L2)	CHN (L3)	KBP (L4)	LDN (L5)	NLR (L6)	NWG (L7)	
Kharif, 2017	NWGR-13017	14.80	17.10	21.10	10.30	29.80	5.60	9.60	15.47
	W 1263 (RC)	12.80	15.10	26.60	13.70	17.90	6.60	13.00	15.10
	TN 1 (SC)	18.90	19.80	52.20	10.90	40.80	14.40	17.30	24.90

RC- Resistant check, SC- Susceptible check, Locations (L): PTB-Pattambi (Kerala), KUL-Kaul (Haryana), CHN- Chinsurah (West Bengal), KBP-Kurumbapet (Pondicherry), LDN-Ludhiana (Punjab), NLR-Nellore (Andhra Pradesh), NWG- Nawagam (Gujarat) and MSD-Masodhha (Uttar Pradesh)

Table 3: Performance of NWGR-13017 against leaf folder at Nawagam and Navsari location under State screening trial in natural condition

Year	Location	Reaction of leaf folder (Damage Score, 0-9)		
		NWGR-13017	GR 11 (C)	GR 103 (C)
Kharif, 2015	Nawagam	1	3	3
	Navsari	1	3	1
Kharif, 2016	Nawagam	1	3	3
	Navsari	3	3	1
Range		1-3 (R to MR)	3 (MR)	1-3 (R to MR)

R-Resistant and MR-Moderately Resistant (Anonymous, 2013 SES damage scale (0-9)

recorded to calculate per cent damage in each genotype. The results presented Table 2 on the leaf folder screening trial (LFST) carried out during *kharif*, 2016 and 2017 revealed that the genotype NWGR-13017 was found promising and outstanding perform against leaf folder in five and seven different locations across the India in both the years, respectively. In *kharif* 2016, lowest mean per cent damage leaves 37.58 were recorded in genotype NWGR-13017. While, maximum damaged leaves (46.82%) were recorded in susceptible check TN-1. During *kharif*, 2017 the genotype NWGR-13017 was found promising and recorded 15.47 per cent damaged leaves in all the locations including Nawagam. However, susceptible check TN-1 had maximum per cent damaged leaves (24.90) in *kharif*, 2017. The state screening trial data presented in Table 3 revealed that the genotype NWGR-13017 was found resistant against leaf folder in both Nawagam and Navsari locations during *kharif*, 2015 and 2016. This indicated that the genotype NWGR-13017 showed resistant against leaf folder at most of the locations across the India. Therefore, it can be used as a donor parent in breeding programme for development of leaf folder resistant variety. Moreover, in the 53rd ARGM on 13- 16 April, 2018 at IIRR, Hyderabad, reported that the rice genotype NWGR-13017 developed from Nawagam was identified as promising against leaf folder and recommended for utilization as donor in breeding programme (Anonymous, 2018).

References

Anonymous (2013). 5th Edition, June, Standard Evaluation System for Rice (SES). P.O. Box 933, 1099 Manila, Philippines, pp 31-32.

Anonymous (2018) Draft proceeding of 53rd Annual Rice Research Group Meeting, IIRR, Hyderabad, p.73.

5. Rahaspunjar (IC-575321; AC 42138) (IC575321; INGR21116), a rice (*Oryza sativa* L.) germplasm with tolerance to salinity stress, stagnant flooding (both fresh and saline water) and high anaerobic germination potential

Koushik Chakraborty, Krishnendu Chattopadhyay, BC Marndi, BC Patra*, Padmini Swain and RK Sarkar

ICAR-National Rice Research Institute, Cuttack-753006, Odisha, India

**Email: bcpatracrri@yahoo.com*

Introduction

The genotype 'Rahaspunjar/Rashpanjor' (IC-575321; AC 42138), a landrace from coastal Odisha, was found tolerant to multiple abiotic stresses like salinity at early vegetative stage (Chattopadhyay *et al*. 2014; Singh & Sarkar 2014), stagnant flooding at late vegetative

(tillering) and reproductive stages (Panda *et al.* 2019; Prusty *et al.* 2018; Pradhan *et al.* 2018; Chakraborty *et al.* 2021), and germination stage oxygen deficiency (*anaerobic* germination) during germination stage (Senapati *et al*. 2019). It was found to be moderately tolerant to vegetative stage salinity stress (12 dS m^{-1}) with an SES score of '5' and Na^+/K^+ ratio of 1.29, 1.01 and 1.11 in root, shoot and total plant, respectively (Chattopadhyay *et al.* 2014). Physiological evaluation showed that Rahaspunjar is tolerant to both fresh and saline water flooding at late vegetative to reproductive stage, which means it has a rare ability to withstand the individual stress of stagnant flooding as well as combined stresses of salinity and stagnant flooding (Chakraborty *et al.* 2021).

Morpho-agronomic Characteristics

Rahaspunjar was found to be medium yielder (3.5 t/ha). The average maturity duration was found long (140-150 days). It has tall (145–155 cm) plant type. It has long well exserted panicle with long bold grain type. Multi-locational testing conducted at 11

AICRIP centers in 2019-2020, showed that Rahaspunjar is tolerant to vegetative stage salinity stress (12 dS m^{-1}), with <15% reduction in germination percentage and <30% reduction in overall plant dry biomass. This genotype was also found to be tolerant to anaerobic germination (AG) condition with the lowest reduction in germination percentage over control (average of all locations) and <20% reduction in seedling vigour under anaerobic stress imposed as 10 cm of standing water at the time of germination.

Associated Characters and Cultivated Practices

Rahaspunjar is found to possess multiple abiotic stress (salinity, stagnant flooding and anaerobic germination and combined stresses of saline water flooding) tolerance in multi-season and multi-locational testing. Further, global comparative transcriptomic study and morpho-physiological and anatomical evaluation revealed a unique mechanism of tolerance against combined stresses of salinity and stagnant flooding (waterlogging) in Rahaspunjar. We found, the presence of well-developed preformed constitutive aerenchyma due to the coordinated action of *RBOH* (*respiratory burst oxidase homolog*) and MT (*metallothionein*) gene homologs, is the key determining factor towards tolerance to combined stress of salinity and stagnant flooding (Chakraborty *et al.* 2021). This valuable rice germplasm is found to have stable source for multiple abiotic stress tolerance. This genotype is tolerant to individual stresses like salinity (both at early seedling and reproductive stages), stagnant flooding (at tillering and reproductive stages) and combined stresses of salinity and stagnant flooding as encountered during saline water flooding. Additionally, this genotype also possesses considerably high anaerobic germination (AG) potential as over the years and in different locations.

Reference

Chakraborty K, Ray S, Vijayan J, Molla KA, Nagar R, Jena P, Mondal S, Panda BB, Shaw BP, Swain P, Chattopadhyay K, Sarkar RK (2021) Preformed aerenchyma determines the differential tolerance response under partial submergence imposed by fresh and saline water flooding in rice. *Physiol. Plant.* **173(4):** 1597-1615.

Chattopadhyay K, Nath D, Mohanta RL, Marndi BC, Singh DP, Singh on (2014). Morpho-physiological and molecular variability in salt tolerant and susceptible popular cultivars and their derivatives at seedling stage and potential parental combinations in breeding for salt tolerance in rice. *Cereal Res. Commun.* **43(2):** 236-48.

Panda D, Ray A, Sarkar RK (2019). Yield and photochemical activity of selected rice cultivars from Eastern India under medium depth stagnant flooding. *Photosynthetica,* **57(4):** 1084-93.

Pradhan B, Chakraborty K, Prusty N, Mukherjee AK, Chattopadhyay K, Sarkar RK (2018). Distinction and characterisation of rice genotypes tolerant to combined stresses of salinity and partial submergence, proved by a high-resolution chlorophyll fluorescence imaging system. *Funct. Plant Biol,* **46(3):** 248-61.

Prusty N, Pradhan B, Chattopadhyay K, Patra BC, Sarkar RK (2018). Novel Rice (*Oryza sativa* L.) Genotypes Tolerant to Combined Effect of Submergence and Salt Stress. *Indian J. Plant Genet. Res.,* **31(3):** 260-269.

Senapati S, Kuanar SR, Sarkar RK (2019). Anaerobic Germination Potential in Rice (*Oryzasativa* L.): Role of Amylases, Alcohol deydrogenase and Ethylene. *J. stress physiol. biochem.,* 15(4).

Singh DP, Sarkar RK (2014). Distinction and characterization of salinity tolerant and sensitive rice cultivars as probed by the chlorophyll fluorescence characteristics and growth parameters. *Funct. Plant Biol.***41(7):** 727-736.

6. Remeni Pokkali (AC 41585) (IC640648; INGR21117), a rice (*Oryza sativa var.* indica) germplasm with tolerance to salinity at vegetative stage (12 dS m-1) and tolerance to salinity at reproductive stage (8 dS m-1)

Krishnendu Chattapadhyay, Koushik Chakraborty, BC Marndi, Padmini Swain and BC Patra*

ICAR-National Rice Research Institute, Cuttack-753006, Odisha, India

**Email: bcpatracrri@yahoo.com*

Introduction

Rice grown in the coastal saline areas of Kerala under Pokkali and Kaipad system of cultivation. Pokkali refers to a system of rice cultivation which is characterized by prolonged partial flooding, and farmers alternate rice cultivation with shrimp farming. The rice geramplam and pureline selection from the traditional cultivars and landraces in this region are found to have single or multiple abiotic stress tolerance. Earlier a *Saltol*-QTL for salinity tolerance at seedling stage was identified from one of the accessions of Pokkali and introgressed in high yielding background. But researchers are still searching for donors for the reproductive stage salinity tolerance which can introduce QTLs for substantial reduction in yield penalty under salinity stress. This accession of Pokkali (AC41585) was collected from the Village Kainegeri, Dist. Alappuzha in Kerala. In repeated evaluation under control condition with salinity stress, EC= 12 dSm-1, it was found tolerant (SES score- 3) to salinity at early vegetative stage. Most importantly it was found also tolerant (<25% yield reduction) to salinity stress at reproductive stage.

Morpho-agronomic characteristics

This germplasm has intermediate seedling vigour. Coleoptile is green. Basal leaf sheath colour is green. Leaf blade colour is green. Leaf pubescence is medium. It is medium maturity duration (110-125 days). It is tall (120-135 cm) and having medium tillering ability (6). It has long (28 cm), dropping and well exerted panicle. It has long bold grain with 30 g test weight. Grain yield is around 2 t/ha under moderate (6-8 dSm-1) salinity stress condition.

Associated characters and cultivation practices

Pokklai accession AC 41585 was found tolerant with stable growth both at the seedling and reproductive stages over the years and environments. AC 41585 was found to be a salinity tolerant genotype based on low reduction (<25%) of grain yield and yield component traits under salinity stress (EC= 8 dSm^{-1}) at reproductive stage (Chattopadhyay *et al.* 2013a). Higher K^+ concentration, lower Na+ concentration and lower Na^+/K^+ ratio in flag leaf as compared to susceptible genotypes was observed in reproductive stage salinity tolerance genotype, Pokkali (AC 41585) (Chattopadhyay *et al.* 2018). It was further explained that this genotype, a potential Na+ excluder, managed to sequester higher Na+ load in the roots with little upward transport as evident from greater expression of HKT1 and HKT2 transporters (Chakraborty *et al.* 2019, ICAR-NRRI Annual Report 2018-19). Using mapping population from IR64/AC41585, 9 multi-environmental consistent QTLs for reproductive stage salinity tolerance with 17-42% phenotypic variances were detected (Chattopadhyay *et al.* 2020a). AC 41585 was also detected with salinity (EC= 12 dSm^{-1}) tolerant at seedling stage (SES score= 3) and 28 QTLs related to photosynthesis and 2 QTLs for stress susceptibility index for Na^+/K^+ ratio were detected for seedling stage salinity tolerance (Chattopadhyay *et al.* 2020b).

References

Chakraborty K, Chattaopadhyay K, Nayak L, Ray S, Yeasmin L, Jena P, Gupta S, Mohanty SK, Swain P and Sarkar RK (2019). Ionic selectivity and coordinated transport of Na+ and K+ in flag leaves render differential salt tolerance in rice at the reproductive stage. *Planta*, *https://doi.org/10.1007/s00425-019-03253-9*.

Chattopadhyay K, Nath D, Das G, Mohanta RL, Marndi BC, Singh DP, Sarkar RK and Singh ON. (2013a). Phenotyping and QTL linked marker based genotyping of rice lines with varying level of salt tolerance at flowering stage. *Indian J. Genet. Plant Breed,* ***73*****(4):** 434-437.

Chattopadhyay K, Nath D, Mohanta RL, Bhattacharyya S, Marndi BC, Nayak AK, Singh DP, Sarkar RK and Singh ON (2013b). Diversity and validation of microsatellite markers in *Saltol*

QTL region in contrasting rice genotypes for salt tolerance at the early vegetative stage. *Aus. J. Crop Sci,* **8 (3):** 356-362.

Chattopadhyay K**,** Nayak AK, Marndi BC, Poonam A, Chakraborty K and Sarkar RK (2018). Novel screening protocol for precise phenotyping of salt-toleranceat reproductive stage in rice. *Physiol. Mol. Biol. Plants*, *https://doi.org/10.1007/s12298-018-0591-7*.

Chattopadhyay K, Mohanty SK, Vijayan J, Marndi BC, Molla KA, Chakraborty K, Ray S and Sarkar RK (2020a). Genetic dissection of component traits for salinity tolerance at reproductive stage in rice. *Plant Mol. Biol. Rep*, *https://doi.org/10.1007/s11105-020-01257-4*.

Chattopadhyay K, Vijayan J, Ray A, Chakraborty K, and Sarkar RK (2020b). Additive main effect and digenic epistatic quantitative trait loci for chlorophyll fluorescence traits influencing salt tolerance at seedling stage in rice. *Photosynthetica,* 58(SI):410-422, *doi: 10.32615/ps.2020.008*

ICAR-NRRI Annual report, 2018-2019.

7. IET25443 (IC640649; INGR21118), a rice (*Oryza sativa* L.) germplasm with high Zn and Fe concentration in polished grain

CN Neeraja*, LV Subba Rao, V Ravindra Babu, RM Sundaram, D Sanjeeva Rao, P Madhu Babu, K Suman and V Jaldhani

ICAR-Indian Institute of Rice Research, Rajendranagar-500030, Hyderabad, Telangana, India.

**Email: cnneeraja@gmail.com*

IET 25443 (RP 4993-300-22-18-1-4-1) is an inbred line derived from BPT 5204/ Chittimutyalu. In the studies of Rice Biofortification, IET 25443 was identified as promising line in terms of polished rice grain Fe and Zn content & Protein content. It is a medium duration line with 105-109 days to 50% flowering and possesses short bold (SB) grain

Table 1: Agro-morphological, yield and grain micronutrient characters of IET 25443

S. No.	Trait	Value
1	Plant Height (cm)[a]	94
2	Tiller Number (Per Hill)	10
3	Productive Tiller Number (Per Hill)	9
4	Days to 50% Flowering [a]	105-108
5	Panicles per Square meter [a]	284-317
6	Single Plant Yield (grams)	14.6
7	1000 grain weight (grams)	17.2
8	Grain Yield (Kg/ha)	4331
9	Grain Fe (ppm) in Polished Rice [a]	3.36
10	Grain Zn (ppm) in Polished Rice [a]	22.6
11	Protein (%) in Polished Rice [a]	7.96
12	Total Ash (%) [b]	17.8
13	Crude Fiber (%) [b]	43.4
14	IVOMD (%) [b]	45.6

a - Based on the data of AICRIP trials; b – Based on the data provided by NIANP, Bengaluru.

type (Table 1). It was evaluated in AICRIP Biofortification trials during 2015, 2016 and 2017 across the locations (IIRR Progress Report, 2016; 2017; 2018). The overall mean grain yield was noted as 4331 Kg. ha^{-1}. During, IVT-Biofortification trial (2015), this culture was ranked 2nd in Kerala state with 6837 Kg/ha grain yield. In addition, this culture was ranked 1st in UP (6942 kg/ha) and 5th in Maharashtra (4210 kg/ha) during AVT-1 Biofortification trial, 2016. In the third year of testing *i.e.,* AVT-2 Biofortification trial, the proposed culture, IET 25443 ranked 2nd in Zone III (5197 Kg/ha) and indicated 10% yield advantage over BPT 5204. In West Bengal it was the first ranking entry (5902 Kg/ha) with 22.6% and 17.8% yield gain over IR 64 and BPT 5204 respectively. In Zone VII, it recorded mean grain yield of 4864 kg/ha and state wise it stood at 3rd rank in AP (5584 Kg/ha) with 9.1% and 9.4% yield improvement over IR 64 and BPT 5204 respectively. In addition to the yield advantage, IET25443 has holds the notable grain micronutrient and protein content. The mean grain Fe and Zn concentration was noted as 7.96 ppm and 22.6 ppm respectively [(IIRR Progress Report, 2016; 2017; 2018), (Table 5 of Sanjeeva Rao *et al.* 2020)]. In addition, it has the protein concentration of 7.96%.

The identified genetic stock, IET25443 comprises 22.6 ppm Zn content, 3.36 ppm Fe content and 7.96% Protein in Polished rice grain (Table 2). It can be used as a potential donor in breeding program in rice bio-fortification studies.

Table 2: Data of IET 25443 under AICRIP trials during 2015, 2016 and 2017

	_Fe (ppm)	*Zn (ppm)*	*Protein (%)*	*Grain Yield (kg/ha)*	*Days to 50% Flowering*	*Plan Height (cm)*	*Panicles/m2*
2015 (IVT-Biofortification)	3.6 (14)	20.4	8.14	3629 (13)	109 (18)	94 (18)	284 (18)
2016 (AVT 1- Biofortification)	4.0 (16)	22.5 (15)	7.92 (4)	4660 (12)	105 (24)	94 (23)	317 (23)
2017 (AVT 2- Biofortification)	2.5 (18)	25 (18)	7.83 (8)	4728 (13)	108 (24)	91 (24)	285 (24)
Mean	3.36	22.6	7.96	4339	107	93	295

Values within parenthesis indicates the number locations tested during AICRIP trials. (IIRR Progress Report, 2016; 2017; 2018)

References

ICAR-Indian Institute of Rice Research, (2016). Progress Report 2015, Vol.1, Varietal Improvement. All India Coordinated Rice Improvement Project. ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad – 500 030, T.S, India *www.icar-iirr.org*

ICAR-Indian Institute of Rice Research, (2017). Progress Report 2016, Vol.1, Varietal Improvement. All India Coordinated Rice Improvement Project. ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad – 500 030, T.S, India *www.icar-iirr.org*

ICAR-Indian Institute of Rice Research, (2018). Progress Report 2017, Vol.1, Varietal Improvement. All India Coordinated Rice Improvement Project. ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad – 500 030, T.S, India *www.icar-iirr.org*

Sanjeeva Rao D, Neeraja CN, Madhu Babu P, Nirmala B, Suman K, Rao LV, Surekha K, Raghu P, Longvah T, Surendra P and Kumar R (2020). Zinc biofortified rice varieties: challenges, possibilities, and progress in India. *Frontiers in nutrition*, 7:26.

8. DH-1 (IC640653; INGR21119), a wheat (*Triticum aestivum* L.) germplasm with resistance to yellow and brown rust in seedling stage adult plant stage

Madhu Patial[1*], Harinder Kumar Chaudhary[2], Dharam Pal[1], SC Bhardwaj[3], OP Gangwar[3] and Kallol Kumar Pramanick[1]

[1]*ICAR-IARI Regional Station, Shimla-171004, Himachal Pradesh, India*

[2]*CSK Himachal Pradesh Agriculture University, Palampur-176061, Himachal Pradesh, India*

[3]*ICAR-IIWBR, Regional Station, Flowerdale, Shimla-171002, Himachal Pradesh, India*

[*]*Email: mcaquarian@gmail.com*

DH-1 is wheat (*Triticum aestivum* L.) doubled haploid line resistance against all the prevailing pathotypes of stripe and leaf rust in seedling stage (except for *77-5* race of leaf rust) and is also resistant to both the rust in Adult Plant stage (Table 1 and 2). The material is developed following *Imperata cylindrica* mediated chromosomal elimination technique by crossing wheat F1s (developed by crossing spring wheat genotype HS542 with winter wheat genotype China 84-40022) with *Imperata cylindrica* at ICAR-IARI Regional Station, CHC, Amartara Cottage, Tutikandi Center, Shimla (H.P.). This wheat genetic stock is developed *via Imperata cylindrica* mediated chromosomal elimination technique and have characteristic features of both winter and spring wheat genotypes, thereby increasing the variability in wheat.

DH-1 has semi-spreading growth habit with late maturity (194 days) under Northern Hill conditions. The distinguish features of DH-1 are presence of scurs, green leaves, erect flag leaf attitude, tapering ear shape with white colour during maturity, straight peduncle attitude and thousand grain weight of 36 grams.

The parent HS542 has awns and China 84-40022 is awnless but the developed DH-1 has scurs. The grain colour of DH-1 is similar to HS542 *i.e* amber, while China-84 has reddish grain colour. The genetic stocks namely FLW3 (UP2338*3 /China84-40022) (Datta *et al.*, 2012) and FLW22 (developed through double cross between WH542/*Lr28* and WH542/China84- 40022), developed by ICAR-IIWBR, Regional Station, Flowerdale, Shimla, also contains China 84-40022 as one of the parents (Bhardwaj *et al.*, 2015) and has reported to be resistant to yellow rust and contains *Yr* China 84 genes which has been found to be resistant to all the races of yellow rust till date.

The role of China 84 as resistant sources has been identified

Table 1: The seedling resistant response of DH-1, parents and Agra Local to yellow and brown rust and gene postulation

Genotypes	*Seedling screening*									*Postulated gene*
	Yellow Rust									
	110S119	110S247	238S119	78S84	110S84	111S68	46S119	T	P	
AL	3+	3+	3+	3+	3+	3+	3+	3+	3+	-
HS542	3+	33+	3+	;	3-	;	;	0;	;	*Yr2+*
China84-40022	0;	0;	;CN	0;	0;	0;	0;	0;	0;	*Yr China+*
DH-1(HS 542 / China 84-40022)	0;	;	;-	0;	;	0;	0;	0;	0;	*Yr9+*

Genotypes	Leaf Rust																	Postulated gene
	11	12-5	12-7	12A	77	77-1	77-2	77-5	77-8	77-10	77A-1	104-2	107-1	108-1	162-1	77-9	104-1	
AL	3+	3	3	3	3	3	3	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	-
HS542	0;	0;	;1	2	3	;	3	3+	;	0;	3+	2	2	2	3+	3+	3+	*Lr13 +*
China84-40022	;1	3	3+	33+	2	3+	3+	3	;1	;	3+	2	2	2	3+	3+	3+	*Lr13 +*
DH-1(HS 542 / China 84-40022)	0;	;1	;	0;	0;	;	0;	3+	;	0;	0;	2	;1	;1	0;	;	0;	*Lr26 + 23 + 1+*

Table 2: Infection type in DH-1, parents and Agra Local to yellow and brown rust at Adult Plant stage

Genotype	*APR (Yellow Rust) Dhaulakuan*	*APR (Brown Rust) Dhaulakuan*	*APR (Yellow Rust) Bajaura*	*APR (Yellow Rust) Shimla*
AL	30S	40S	40S	20S
HS542	10S	10S	10S	5S
China 84-40022	R	5S	R	R
DH 1 (HS 542 / China 84-40022)	R	R	R	R

by many scientists (Datta *et al.*, 2012). The DH-1 also has China 84-40022 as one of the parents and is a resistant source to all the races of yellow and brown rust.

The molecular screening with gene specific markers highlighted the presence of adult plant leaf rust resistant gene *Lr34* along with *Lr26 and Lr32* genes. The rust resistance gene pool present in DH-1 would prove to be an important source for developing potential rust resistant genotypes and/ or serve as potent donor for creating new usable variability against different pathotypes of rust in wheat improvement programme of India having characteristics from winter gene pool also.

References

Datta D, Prashar M, Bhardwaj SC, Singh S, Das SP and Kumar A. (2012). Deciphering the genetic basis of stripe rust resistance of exotic winter wheat cultivars and their utilization in pre-breeding. *Afr. J. Agric. Res.*, **7(49):** 6544-6549.

Bhardwaj SC, Gangwar OP, Prasad P and Khan H. (2015). Mehtansis. In: ICAR-indian Institute of wheat and barley research, regional station, Flowerdale, Shimla, H.P., 9p.

9. QLD121 (IC640670; INGR21120), a wheat (*Triticum aestivum* L.) germplasm with low grain hardness index and low sedimentation value

Gopalareddy K*, D Mohan, BS Tyagi, Hanif Khan, CN Mishra, Sewa Ram, Vanita Pandey, OP Gupta, Ishwar Singh, Mamrutha HM, Arun Gupta, Gyanendra Singh and GP Singh

ICAR-Indian Institute of Wheat and Barley Research, Karnal-132001, Haryana, India

Email: gopalareddy.k@icar.gov.in

Grain hardness is an important trait in wheat quality with a profound effect on milling, baking and end-use qualities of wheat. It is common to differentiate soft and hard wheat in the world trade for product specific utility. Soft wheat is more friable, requires less energy to mill, and produces flours and meals with finer particles and lower starch damage, which are suitable for cake and biscuit production. Soft grain textured wheat produces tender and larger biscuits. QLD 121 was developed at ICAR-Indian Institute of Wheat and Barley Research (ICAR-IIWBR) by crossing DPW621-50 / PBW550. The genotype was evaluated at 15 different locations across the country for different agro- morphological traits in Quality Component and Wheat Bio-fortification Nursery (QCWBN) and the results are presented in Table 2. However, due to high heritability of grain hardness index, only 06 centres comprising all the major wheat growing zones of the country has been analysed for grain hardness index. QLD 121 was found to be superior with 21 grain hardness index over the locations to all the tested genotypes and checks including soft grain check variety HS 490 (Table 1). Although, grain hardness of parentage of QLD 121 was hard, transgressive sergeants were appeared for soft grain. QLD 121 recorded the lowest grain hardness index in all the tested centres compared to the best check variety (HS 490) for low grain index. QLD 121 recorded lowest grain hardness index of 24, 14, 17, 18, 22, and 27 respectively at Karnal, Delhi, Kanpur, Indore, Vijapur, and Pune centres, whereas, the soft grain check variety HS 490 reported 37, 29, 23, 39, 40, and 37. The QCWBN nursery was also tested for rust reaction in Initial Plant Pathological Nursery (IPPSN) and the results are presented in (Table 2). The genotype is resistant to all the three rusts (stripe, leaf and stem). QLD 121 also has the lowest sedimentation value compared to all the checks. Low grain hardness index and sedimentation value are very important factors to obtain high spread factor of biscuit and better biscuit quality. Thus, QLD 121 would be a potential source to be utilized in future breeding programs to develop high yielding, disease resistant bread wheat varieties suitable for better biscuit making.

Table 1: Grain hardness index of QLD121 at 6 locations during 2020-21

Zone	*Location*	*Genotype*	*Check Varieties*					
		QLD 121	*HS490 (Soft Check)*	*WB02*	*DBW187*	*HD3226*	*GW322*	*DDW47*
NWPZ	Karnal	24	37	79	79	85	83	91
	Delhi	14	29	70	79	83	80	85
NEPZ	Kanpur	17	23	80	75	78	70	81
CZ	Indore	18	39	69	75	80	88	88
	Vijapur	22	40	76	78	84	86	90
PZ	Pune	27	37	75	73	76	87	89
Mean (National)		21	35	75	77	81	82	87

Table 2: Agro-morphological, disease and quality traits of QLD 121 at 15 locations during 2020-21

Trait_Genotype	*QLD 121*	*HS490 (Soft Check)*	*WB02*	*DBW187*	*HD3226*	*GW322*	*DDW47*
Agro-Morphological Traits							
Grain yield (q/ha)	53.3	45.9	45.8	54.6	56.7	51.4	45.8
Plant Height (cm)	93	101	86	93	98	92	91
Heading (days)	81	79	72	75	80	76	80
Thousand Kernel weight (gm)	41	41	39	44	40	40	39
Disease Reaction (IPPSN)							
Stripe Rust (ACI)	11.7	6.4	-	3.2	-	36	3.8
Leaf Rust-North (ACI)	3.5	3.6	-	5.9	-	8.6	3.4
Leaf Rust-South(ACI)	5.2	4.1	-	9.5	-	7.3	7.7
Stem Rust (ACI)	1.8	11.8	-	12	-	8.3	3.5
Quality Traits							
Grain Iron (ppm)	36.8	37.0	40.7	39.6	39.5	36.6	38.1
Grain Zinc (ppm)	42.7	41.0	44.3	37.0	41.3	41.4	43.8
Grain Protein Content (%)	12.4	11.5	13.9	12.1	12.9	10.9	12.1
Sedimentation Value (ml)	38.8	39.7	60.7	57.3	59.6	38.6	32.5
Hectolitre Weight (Kg/hl)	75.7	74.7	77.3	78.6	78.3	76.8	78.1

10. QLD120 (IC640671; INGR21121), a wheat (*Triticum aestivum* L.) germplasm with low grain hardness index and high nutritional value

Gopalareddy K*, D Mohan, BS Tyagi, Hanif Khan, CN Mishra, Sewa Ram, Vanita Pandey, Ishwar Singh, Mamrutha HM, Rinki, Gyanendra Singh and GP Singh

ICAR-Indian Institute of Wheat and Barley Research, Karnal-132001, Haryana, India

Email: gopalareddy.k@icar.gov.in

Grain hardness is an important trait in wheat quality with a profound effect on milling, baking and end-use qualities of wheat. It is common to differentiate soft and hard wheat in the world trade for product specific utility. Soft wheat is more friable, requires less energy to mill, and produces flours and meals with finer particles and lower starch damage, which are suitable for cake and biscuit production. Soft grain textured wheat produces tender and larger biscuits. QLD 120 was developed at ICAR-Indian Institute of Wheat and Barley Research (ICAR-IIWBR) by crossing PBW343/VL738// PBW611/3/39th IBWSN1108 (SUNSU/CHIBIA)/DBW17. The genotype was evaluated at 15 different locations across the country for different agro-morphological traits in Quality

Table 1: Grain hardness index of QLD 120 and check varieties at 6 locations during 2020-21

Zone	*Location*	*Genotype*	*Check Varieties*				
		QLD 120	*HS490 (Soft Check)*	*WB02*	*DBW187*	*HD3226*	*GW322*	*DDW47*
NWPZ	Karnal	44	37	79	79	85	83	91
	Delhi	19	29	70	79	83	80	85
NEPZ	Kanpur	24	23	80	75	78	70	81
CZ	Indore	30	39	69	75	80	88	88
	Vijapur	29	40	76	78	84	86	90
PZ	Pune	28	37	75	73	76	87	89
Mean (National)		29	35	75	77	81	82	87

Table 2: Agro-morphological, disease and quality traits of QLD 120 and check varieties during 2020-21

Trait	*Genotype*	*Check Varieties*					
	QLD 120	*HS490 (Soft Check)*	*WB02*	*DBW 187*	*HD 3226*	*GW 322*	*DDW 47*
Agro-Morphological Traits							
Grain yield (q/ha)	52.1	45.9	45.8	54.6	56.7	51.4	45.8
Plant Height (cm)	90	101	86	93	98	92	91
Heading (days)	78	79	72	75	80	76	80
Thousand Kernel weight (gm)	42	41	39	44	40	40	39
Disease Reaction (IPPSN)							
Stripe Rust (ACI)	6.4	6.4	-	3.2	-	36	3.8
Leaf Rust-North (ACI)	2.6	3.6	-	5.9	-	8.6	3.4
Leaf Rust-South(ACI)	6.0	4.1	-	9.5	-	7.3	7.7
Stem Rust (ACI)	3.6	11.8	-	12	-	8.3	3.5
Quality Traits							
Grain Iron (ppm)	41.2	37.0	40.7	39.6	39.5	36.6	38.1
Grain Zinc (ppm)	47.2	41.0	44.3	37.0	41.3	41.4	43.8
Grain Protein Content (%)	13.1	11.5	13.9	12.1	12.9	10.9	12.1
Sedimentation Value (ml)	42.1	39.7	60.7	57.3	59.6	38.6	32.5
Hectolitre Weight (Kg/hl)	77.6	74.7	77.3	78.6	78.3	76.8	78.1

Component and Wheat Bio-fortification Nursery (QCWBN) and the results are presented in Table 2. However, due to high heritability of grain hardness index, only 06 centres comprising all the major wheat growing zones of the country has been analyzed for grain hardness index. QLD 120 was found to be superior with 29 grain hardness index over the locations to all the check varieties including soft grain check variety HS 490 (Table 1). QLD 120 recorded the lowest grain hardness index in all the tested centres compared to the best check variety (HS 490) for low grain index. QLD 120 recorded lowest grain hardness index of 44, 19, 24, 30, 29, and 28 respectively at Karnal, Delhi, Kanpur, Indore, Vijapur, and Pune centres, whereas, the soft grain check variety HS 490 reported 37, 29, 23, 39, 40, and 37. The QCWBN was also tested for rust reaction in Initial Plant Pathological Nursery (IPPSN) and the results are presented in Table 2. The genotype is highly resistant to all the three rusts (stripe, leaf and stem). QLD 120 is also having lowest sedimentation value compared to all the checks. Low grain hardness index and sedimentation value are very important factors to obtain high spread factor of biscuit and better biscuit quality. Thus, QLD 120 would be a potential source to be utilized in future breeding programs to develop bread wheat varieties suitable for better biscuit making.

11. QLD118 (IC640672; INGR21122), a wheat (*Triticum aestivum* L.) germplasm with very high grain zinc and high grain yield

Gopalareddy K*, D Mohan, BS Tyagi, Vikas Gupta, Charan Singh, PL Kashyap, Ishwar Singh, Mamrutha HM, UR Kamble, Gyanendra Singh and GP Singh

ICAR-Indian Institute of Wheat and Barley Research, Karnal-132001, Haryana, India

**Email: gopalareddy.k@icar.gov.in*

Worldwide over 2 billion people suffer from iron (Fe), zinc (Zn) and/or other (multiple) micronutrient deficiencies. In India, 48% of children under the age of 5–10 years have zinc/iron or some other micronutrient deficiency. Zinc is one of the important micronutrient for normal growth and development. QLD 118 was developed at ICAR-Indian Institute of Wheat and Barley Research (ICAR-IIWBR) by crossing 43rd IBWSN1137 (MINO/898.97)/43rd IBWSN1049 (WHEAR/SOKOLL). The genotype was evaluated at 15 different locations across the country for different agro-morphological traits in Quality Component and Wheat Bio-fortification Nursery (QCWBN) and the results are presented in Table 2. However, the hand threshed grains of 10 centres in QCWBN comprising all the major wheat growing zones

Table 1: Grain iron and zinc concentration of QLD 118 and check varieties at 10 locations during 2020-21

Zone	Location	Genotype		Check Varieties											
		QLD 118		HS490		WB02		DBW187		HD3226		GW322		DDW47	
		Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	38.3
NWPZ	Karnal	33.5	37.3	32.3	35.4	35.1	36.9	31.0	35.7	32.5	35.0	33.6	34.6	35.0	38.7
	Hisar	45.3	46.0	43.13	37.3	44.4	42.8	35.8	40.9	42.1	40.5	40.1	35.1	48.2	37.4
	Delhi	70.3	46.2	57.6	37.5	59.1	39.9	48.7	39.3	53.3	40.0	52.4	37.1	59.9	41.9
	Ludhiana	49.3	35.5	35.8	38.1	37.4	41.6	33.7	44.0	34.4	37.5	35.5	37.0	38.0	32.8
NEPZ	Kanpur	40.5	35.1	37.8	32.4	38.0	34.0	32.3	32.0	40.6	35.2	38.4	31.3	42.0	35.9
	Varanasi	39.3	35.5	33.3	34.0	34.6	38.0	29.2	37.0	33.2	37.9	34.2	34.9	36.9	39.3
CZ	Indore	55.1	43.8	46.1	37.0	51.1	40.2	42.8	41.3	50.7	42.7	47.4	38.0	46.6	39.7
	Vijapur	52.2	42.0	48.0	41.0	56.1	46.5	44.1	43.3	49.6	45.5	49.5	42.4	50.4	39.3
PZ	Pune	57.6	44.6	45.1	40.2	50.0	44.2	42.4	40.6	46.7	40.6	47.8	38.0	47.4	38.3
	Dharwad	39.5	44.1	31.3	37.1	37.6	43.1	29.9	41.7	31.2	39.8	34.9	37.7	33.9	38.3
Mean (National)		48.3	41.0	41.0	37.0	44.3	40.7	37.0	39.6	41.3	39.5	41.4	36.6	43.8	38.1

Table 2: Agro-morphological, disease, and quality traits of QLD 118 and check varieties during 2020-21

Trait	Test Genotype	Check Varieties					
	QLD 118	HS490	WB02	DBW187	HD3226	GW322	DDW47
Agro-Morphological Traits							
Grain yield (q/ha)	56.4	45.9	45.8	54.6	56.7	51.4	45.8
Plant Height (cm)	97	101	86	93	98	92	91
Heading (days)	78	79	72	75	80	76	80
Thousand Kernel weight (gm)	41	41	39	44	40	40	39
Disease Reaction (IPPSN)							
Stripe Rust (ACI)	10.8	6.4	-	3.2	-	36	3.8
Leaf Rust-North (ACI)	6.0	3.6	-	5.9	-	8.6	3.4
Leaf Rust-South(ACI)	12.2	4.1	-	9.5	-	7.3	7.7
Quality Traits							
Grain Protein Content (%)	12.5	11.5	13.9	12.1	12.9	10.9	12.1
Sedimentation Value (ml)	59.0	39.7	60.7	57.3	59.6	38.6	32.5
Hectolitre Weight (Kg/hl)	78.4	74.7	77.3	78.6	78.3	76.8	78.1

of the country has been analysed for grain iron and zinc concentration. QLD 118 was found to be superior for grain zinc concentration (48.3 ppm) over the locations to all the check varieties (Table 1). High grain zinc has been incorporated in to the high yielding genetic background through conventional breeding. The QCWBN nursery was also tested for rust reaction in Initial Plant Pathological Nursery (IPPSN) 2020-21 and the results are presented in Table 2. QLD 118 was also resistant to both stripe and leaf rust. Thus, QLD 118 would be a potential source to be utilized in future breeding programs to develop high yielding, disease resistant bread wheat varieties with enhanced grain zinc concentration.

12. QLD122 (IC640673; INGR21123), a wheat (*Triticum aestivum* L.) germplasm with high grain iron and zinc content

Gopalareddy K*, D Mohan, BS Tyagi, Satish Kumar, Amit Sharma, PL Kashyap, Ishwar Singh, Mamrutha HM, Arun Gupta, Gyanendra Singh and GP Singh

ICAR-Indian Institute of Wheat and Barley Research, Karnal-132001, Haryana, India

**Email: gopalareddy.k@icar.gov.in*

Worldwide over 2 billion people suffer from iron (Fe), zinc (Zn) and/or other (multiple) micronutrient deficiencies. In India, 48% of children under the age of 5-10 years have zinc/iron or some other micronutrient deficiency. Zinc is one of the important micronutrient for normal growth and development. QLD 122 was developed at ICAR-Indian

Table 1: Grain iron and zinc concentration of QLD 122 at 11 locations during 2020-21

Zone	Location	Genotype		Check Varieties											
		QLD 122		HS490		WB02		DBW187		HD3226		GW322		DDW47	
		Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn
NWPZ	Karnal	55.5	36.2	35.4	32.3	36.9	35.1	35.7	31.0	35.0	32.5	34.6	33.6	38.3	35.0
	Hisar	41.1	43.6	37.3	43.13	42.8	44.4	40.9	35.8	40.5	42.1	35.1	40.1	38.7	48.2
	Delhi	40.0	63.5	37.5	57.6	39.9	59.1	39.3	48.7	40.0	53.3	37.1	52.4	37.4	59.9
	Ludhiana	39.4	32.9	38.1	35.8	41.6	37.4	44.0	33.7	37.5	34.4	37.0	35.5	41.9	38.0
NEPZ	Kanpur	34.6	40.1	32.4	37.8	34.0	38.0	32.0	32.3	35.2	40.6	31.3	38.4	32.8	42.0
	Varanasi	37.8	34.8	34.0	33.3	38.0	34.6	37.0	29.2	37.9	33.2	34.9	34.2	35.9	36.9
CZ	Indore	47.3	61.1	37.0	46.1	40.2	51.1	41.3	42.8	42.7	50.7	38.0	47.4	39.3	46.6
	Vijapur	47.3	50.1	41.0	48.0	46.5	56.1	43.3	44.1	45.5	49.6	42.4	49.5	39.7	50.4
PZ	Pune	46.6	57.1	40.2	45.1	44.2	50.0	40.6	42.4	40.6	46.7	38.0	47.8	39.3	47.4
	Dharwad	50.4	37.6	37.1	31.3	43.1	37.6	41.7	29.9	39.8	31.2	37.7	34.9	38.3	33.9
Mean (National)		44.0	45.7	37.0	41.0	40.7	44.3	39.6	37.0	39.5	41.3	36.6	41.4	38.1	43.8

Table 2: Agro-morphological, disease and quality traits of QLD 122 at 15 locations during 2020-21

Trait	Test Genotype	Check Varieties					
	QLD 122	HS490	WB02	DBW187	HD3226	GW322	DDW47
Agro-Morphological Traits							
Grain yield (q/ha)	49.5	45.9	45.8	54.6	56.7	51.4	45.8
Plant Height (cm)	95	101	86	93	98	92	91
Heading (days)	83	79	72	75	80	76	80
Thousand Kernel weight (gm)	44	41	39	44	40	40	39
Disease Reaction							
Stripe Rust (ACI)	4.9	6.4	-	3.2	-	36	3.8
Leaf Rust-North (ACI)	2.7	3.6	-	5.9	-	8.6	3.4
Leaf Rust-South(ACI)	4.9	4.1	-	9.5	-	7.3	7.7
Stem Rust (ACI)	3.2	11.8	-	12	-	8.3	3.5
Quality Traits							
Grain Protein Content (%)	13.0	11.5	13.9	12.1	12.9	10.9	12.1
Sedimentation Value (ml)	47.1	39.7	60.7	57.3	59.6	38.6	32.5
Hectolitre Weight (Kg/hl)	76.3	74.7	77.3	78.6	78.3	76.8	78.1

Institute of Wheat and Barley Research (ICAR-IIWBR) by crossing 15th HRWSN286 (MILAN/3/PAT24/ALD//DOVE/BUC)/ CIMMYT165. The genotype was evaluated at 11 centres in Quality Component Screening Nursery (QCSN) comprising all the major wheat growing zones of the country. QLD 122 was found to be superior for grain iron (44 ppm) and zinc (45.7 ppm) over the locations to all the check varieties (Table 1). High grain iron and zinc has been incorporated in to the improved genetic background through conventional breeding. QCWBN nursery has been evaluated at 15 different locations across the country for agro-morphological traits and the results are presented in Table 2. The QCWBN nursery was also tested for rust reaction in Initial Plant Pathological Nursery (IPPSN) and the results are presented in Table 2. QLD 122 is also resistant to all the three rusts (stripe, leaf and stem rust). Thus, QLD 122 would be a potential source to be utilized in future breeding programs to develop high yielding, disease resistant bread wheat varieties with enhanced grain iron and zinc concentration.

13. HD3304 (IC640683; INGR21124), a wheat (*Triticum aestivum* L.) germplasm with very high sedimentation value for greater gluten strength

Anju Mahendru Singh*, Rajbir Yadav and Arvind K Ahlawat

Division of Genetics, ICAR-Indian Agricultural Research Institute, Pusa Campus-110012, New Delhi, India

**Email: anju.singh@icar.gov.in*

Indian wheat varieties have moderately strong gluten, whereas the bread baking industry desires flour with stronger gluten. In the absence of this criteria being met, chemicals for improving the gas retention capacity and gluten strengthening are used in large quantities. Some of the common chemicals used in baking industry are potassium bichromate, calcium peroxide, ascorbic acid etc. Indiscriminate use of these strong oxidizing agents can be harmful for consumer health in the long run.

Therefore, donors with these quality traits are required to be identified/developed and used in breeding programmes. HD3304 is one such germplasm. HD3304 based on its high sedimentation value in station trial was promoted to Quality Component & Wheat Biofortification Nursery where it was evaluated in 12 locations encompassing all the four zones. It gave a mean value of 75 ml which is the highest recorded so far in any year of testing for any genotype (Table 1 & 2). Wheat with high sedimentation value has strong gluten

Table 1: Mean values of four quality traits in the genotype HD3304 tested over12 locations for three years in the Quality Component& Wheat Biofortification Nursery, 2018-19 to 2020-21

Genotype	*Mean Sedimentation value (ml) NWPZ*	*Mean Sed value (ml) NEPZ*	*Mean Sed value (ml) CZ*	*Mean Sed value (ml) PZ*	*Mean Sed value (ml) all locations mean*
HD3304	69	78	76	78	75
HD3086	62	55	57	66	60
WB02	70	75	68	76	72

Table 2: Promising genotypes identified in QCSN 2018-19 for individual quality parameters

Component	*Genotypes*	*Range*		*Best Check*
Protein content (%)	GW20171-596, UP2994	13.8	- 14.6	WB2 (13.5)
Sedimentation value (ml)	HD3304, HD3241, HD3215	73	- 75	WB2 (72)
Grain hardness index (hard wheat)	DBP-17-05 (D), NIAW3284, QLD109	87	- 89	GW 322 & HD 3086 (84)
Grain hardness index (soft wheat)	QLD112	15.0		HS 490 (30)
Hectolitre weight (Kg/hl)	RAJ-4541, QLD109, QBP-18-8, QBP-18-10, KA-1805, UP2994	80.2- 82.5		HD 3086 (78.2)
Grain appearance score (Max. score 10)	RAJ-4541, UP2994, QBP-18-10, QBP-18-19	7.0	- 7.2	MACS 6222 (6.7)
Iron content (ppm)	UP2994, BWL-7800, QBP-17-7	48.1 – 49.0		WB2 (44.8)
Zinc content (ppm)	BWL-7805, Raj-4541, BWL-7800, UP2994	43.5 – 46.9		UP 2672 (39.8)

which is highly desirable for making products such as bread, buns, pizza base etc. HD3304 is additionally, hard grained, has hectoliter weight of 78 Kg/hl and has good yield. In nutshell, HD3304 is suitable for industrial quality and can be used as an elite donor in breeding for superior industrial quality.

14. BHS 481 (BBM 815) (IC640685; INGR21125), a barley (*Hordeum vulgare* L.) germplasm with resistance to seedling resistance to leaf and stripe black rust as well as adult plant resistance to yellow rust

Madhu Patial[1*], Dharam Pal[1], SC Bhardwaj[2], KK Pramanick[1] and OP Gangwar[2]

[1]*ICAR-IARI Regional Station, Shimla-171004, Himachal Pradesh, India*

[2]*ICAR-IIWBR Regional Station, Shimla-171002, Himachal Pradesh, India*

[*]*Email: mcaquarian@gmail.com*

BHS 481 (BBM 815) is a barley (*Hordeum vulgare* L.) line resistant against stripe, leaf and stem rust. It has shown highest degree of resistance against all the prevailing pathotypes of stripe, leaf and stem rust at seedling stage (except moderately resistant to *117-6* race of stem rust) and is also resistant to all the three rusts at adult plant stage. This line is developed following pedigree method of breeding involving crosses between registered barley genetic stock BHS 369 and HBL 113 at ICAR-IARI, Regional Station, CHC, Amartara Cottage, Tutikandi Center, Shimla (H.P.)

BHS 481 (BBM 815) has semi erect growth habit with medium maturity (171 days) under Northern Hill condition. The average yield is 3.63 t/ha under rainfed condition of Northern Hill Zone of All India Co-ordinated trials. For grain yield, BHS 481 (BBM 815) ranked third and was in the 1st Non-Significant Group with average yield of 36.3 q/ha (check variety BHS 400 yield was 36.5 q/ha) when tested in co-ordinated trials under All India Co- ordinated Wheat & Barley Improvement Project during 2019-20. The distinguish features of BHS 481 are six-rowed; hulled; semi-erect growth habit; green leaves; white colour of ear at maturity and thousand grain weight of 41 grams.

References

ICAR-IIWBR 2019. Progress Report of AICRP on Wheat & Barley 2018-19, Barley Improvement. Eds: AS Kharub, Chuni Lal, Dinesh Kumar, Jogendra Singh, Vishnu Kumar, Amit Kumar Sharma, Anil Khippal, Sudheer Kumar, SC Bhardwaj, Poonam Jasrotia, Rekha Malik, Ajay Verma, Satyavir Singh and GP Singh. ICAR-Indian Institute of Wheat and Barley Research, Karnal, India. P. 215.

ICAR-IIWBR 2020. Progress Report of AICRP on Wheat & Barley 2019-20, Barley Improvement. Eds: RPS Verma, AS Kharub, Dinesh Kumar, Chuni Lal, Lokendra Kumar, Jogendra Singh, Amit Kumar Sharma, Anil Khippal, Charan Singh, Sudheer Kumar, SC Bhardwaj, Poonam Jasrotia, Rekha Malik, Ajay Verma, Satyavir Singh and GP Singh. ICAR-Indian Institute of Wheat and Barley Research, Karnal, India. P. 230.

Table 1: Reaction to major diseases

Diseases	*Condition*	*Year*	*Response of Proposed Genetic stock 481 (BBM 815)*	*Page No.*
Stripe Rust (Resistant toyellow rust)	IBDSN (APR)	2018-19	ACI = 0.2 HS = TMS	3.8 of Reference (i)
	NBDSN (APR)	2019-20	ACI = 6.9	3.14 of Reference (ii)
	NBDSN (Seedling)	2019-20	R (Resistant to all races)	3.28 of Reference (ii)
Leaf Rust (Resistant to brown rust)	NBDSN (APR)	2019-20	HS = 0	3.14 of Reference (ii)
	NBDSN (Seedling)	2019-20	R (Resistant to all races)	3.28 of Reference (ii)
Stem Rust (Resistant toblack rust)	NBDSN (APR)	2019-20	5 MS	3.14 of Reference (ii)
	NBDSN (Seedling)	2019-20	R (Resistant to all races) (Except for *117-6* race showing moderately resistant response)	3.28 of Reference (ii)

ACI= Average Coefficient of incidence HS= Highest Score
IBDSN= Initial Barley Disease Screening Nursery NBDSN= National Barley Disease Screening Nursery

15. IML 11 (IC640687; INGR21126), a maize (*Zea mays* L.) germplasm with resistance to *Turcicum* Leaf Blight

Shyam Bir Singh[1*], SI Harlapur[2], N Mallikarjuna[3], R Devlash[4], H Rajashekara[5], KS Hooda[6], Chikkappa G. Karjagi[7], Bhupender Kumar[7], Ramesh Kumar[6], Santosh Kumar[1], Parveen Bagaria[6], Yatish KR[8], BS Jat[6], N Sunil[8], Krishan Kumar[7], RK Kasana[1], Sushil Kumar[1], Shivraj Singh Gangoliya[7] and Sujay Rakshit[6]

[1]*Regional Maize Research and Seed Production Centre, (ICAR-IIMR), Begusarai-851129, Bihar, India*

[2]*Main Agricultural Research Station, University of Agricultural Sciences, Dharwad-580005, Karnataka, India*

[3]*Zonal Agricultural Research Station, V.C. Farm, Mandya, 571405, Karnataka, India*

[4]*CSKHPKV, HAREC, Bajaura, Distt. Kullu –175125, Himachal Pradesh, India*

[5]*Crop Improvement Division, VPKAS, Almora-263601, Uttarakhand, India*

[6]*ICAR-Indian Institute of Maize Research PAU, Campus, Ludhiana-141004, Punjab, India*

[7]*ICAR-Indian Institute of Maize Research Unit, Pusa Campus, New Delhi-110012, India*

[8]*WNC (ICAR-IIMR), Hyderabad, Telangana, India*

[*]*Email: singhsb1971@rediffmail.com*

Introduction

Maize (*Zea mays* L.) is one of the most versatile emerging crops with its highest genetic yield potential among the cereals. The major reason for low productivity of maize in India is losses caused by various biotic and abiotic stresses. Among the various biotic stresses, foliar diseases namely, turcicum leaf blight (TLB) also called northern corn leaf blight caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. (syn. *Heliminthosporium turcicum* Pass.) is of worldwide importance and one of the most important diseases in India. This foliar disease occurs in high humidity and moderate temperature areas. It also results upto 70% loss in the yield. The fungus *E. turcicum* is known to be highly variable in nature (Reddy *et al.* 2013; De-Rossi *et al*., 2015). Host plant resistance is considered as most practical and economically viable method of plant disease management (Wathaneeyawech *et al,* 2015: Hooda *et al*, 2018). The inbred IML 11 is developed at Regional Maize Research and Seed Production Centre (ICAR-IIMR), Begusarai following pedigree method using commercial maize hybris as source material. Under hot spot locations (Dharwad, Mandya, Almora and Bajaura) for TLB, the line IML 11 (IMLSB 334B-1) displayed a mean disease score of 2.6 at par with the resistant check i.e. 2.4 while the susceptible check 7.9.

A set of 237 newly developed maize inbred lines along with resistant (LM 13, CL 4, NAH 1137) and local susceptible (CM 202, CM 600) inbred line as check were evaluated against TLB under artificial epiphytotic conditions. The trial was conducted during kharif season of 2017 and 2018 at TLB hot-spot locations, *viz*., Dharwad, Mandya, Almora and Bajaura. In 2017, the trial was conducted in randomized complete block design whereas in 2018 it was conducted in alpha design. Spore suspension of *E. turcicum* isolates was prepared in lab and sprayed by using atomizer at three to four leaf stage of each and every maize plants twice in an interval of two days to ensure proper inoculation. The disease reaction was recorded by using 1 to 9 scales as suggested by Hooda *et al.* (2018). Disease scoring was commenced from six-eight leaves stage (approximately 45 days after planting) and continued on weekly basis for 6 weeks. The genotypes showing disease score between 0.0–3.0 were considered as resistant (R), 3.1-5.0 as moderately resistant (MR), 5.1- 7.0 as moderately susceptible (MS), >7.0 as susceptible (S). The disease reaction was recorded on five plants in the middle of the row and it was averaged to calculate TLB overall mean disease score of each line. The overall mean TLB score calculated across locations over two years was considered to classify inbred lines into resistant, moderately resistant, moderately susceptible and susceptible inbred lines. Out of 237 inbred lines, 41 inbred lines were resistant with TLB score <3.0, 181 lines were moderately resistance with TLB score 3.1-5.0 and 15 inbred lines were moderately susceptible with TLB score 5.1 to 7.0 (Singh *et al.* 2018). The Inbred lines were also evaluated for maturity and yield traits during rabi-2017-18 and 2018-19 and RMR&SPC, Begusarai. Out of the 41 resistant inbred lines, four best TLB resistant lines along with high yield per se performance as mentioned in Table 1 were identified for registration.

Morpho-agronomic characteristics

IML 11 is a late maturing high yielding line resistant to Turcicum Leaf Blight (disease mean score on the scale of 1-9), short height, medium ear placement, straight attitude of lateral tassel branches, sparse spikelets of tassel, yellow round shaped and flint kernels. It is derived from normal maize segregating progeny by continuous selfing following pedigree breeding method using commercial hybrid as source material.

Associated-agronomic Characteristics

IML 11 is high yielding maize inbred line with yield potential of 28.2 q/ha. This inbred is suitable for cultivation during rabi season. It also has good seed setting to be used as seed parent under commercial seed production. The inbred has straight kernel rows and semi-flint kernels.

Table 1: Disease score for TLB (kharif-2017 &2018), maturity and yield (rabi-2017-18 & 2018-19) of identified newly developed maize inbred lines

S. No	Name of inbred	Another name	TLB score	Reaction	Days to 50% anthesis (rabi)	Days to 75 % dry husk maturity (rabi)	Grain yield (q/ha)	Maturity group
1	IML 11	IMLSB-334B-1	2.6	R	122	158	28.2	Late
2	IML 13	IMLSB-1041-4-1	2.4	R	112	155	36.4	Medium
3	IML 12	IMLSB-446-2	2.5	R	107	147	31.9	Medium
4	IML 21	IMLSB-343-2	2.6	R	125	163	33.1	Late
		Resistant Check (CL 4/ NAH 1137	2.4	R	--	--	--	--
		Susceptible check (CM 200)	7.9	S	--	--	--	--

References

De-Rossi RL, ML Reis, and R Brustalin (2015). Fungal baseline for mycelia sensitivity of *Exserohilum turcicum*, causal agent of northern corn leaf blight. *Summa Phytopathologica,* **41(1):** 25-30.

Hooda KS, PK Bagaria, M Khokhar, H Kaur and S Rakshit (2018). Mass screening techniques for resistance to maize diseases. ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana- 141004, 93pp.

Reddy TR, PN Reddy, and RR Reddy, (2013). Pathogenic variability of isolates of *Exserohilum turcicum*, incitant of leaf blight of maize. *Indian Journal of Plant Protection,* **41(1):** 72-75.

Singh SB, Karjagi CG, Hooda KS, Mallikarjuna N, Harlapur SI, Rajashekara H, Devlash R, Kumar S, Kasana RK, Sonu Kumar, Gangoliya SS and Rakshit S (2018). Identification of resistant sources against turcicum leaf blight of maize (*Zea mays* L.) *Maize Journal,* **7(2):** 64-71.

Wathaneeyawech S, P Sirithunya, and P Smitamana (2015). Collections, isolations, morphological study of *Exserohilum turcicum* and screening resistant varieties of corn to Northern Corn Leaf Blight disease. *International Journal of Agricultural Technology*, **11(4):** 937-952.

16. PRB 903 (IC640691; INGR21127), a barnyard millet (*Echinochloa esculenta (*A. Braun) H.Scholz) germplasm highly resistant to grain smut disease.

Laxmi Rawat[*1], Dinesh C Joshi[2], Vijay Kumar Yadav[3], Bijendra Kumar[5], IK Das[4], Prabhakar Bhatt[6], M Elangovan[4], Amol Vasisth[1] and AK Karnatak[1]

[1]*AICRP on Small Millets, Ranichauri Centre, VCSG Uttarakhand University of Horticulture & Forestry, Bharsar-246123, Uttarakhand, India*

[2]*ICAR- Vivekanand Parvatiya Krishi Anusandhan Sansthan, Almora-263601, Uttarakhand, India*

[3]*ICAR- Indian Grassland and Fodder Research Institute, Jhansi-284003, Uttar Pradesh, India*

[4]*ICAR-Indian Institute of Millets Research, Rajendranagar, Hyderabad-500030, Telangana, India*

[5]*G. B. Pant University of Agriculture & Technology, Pantnagar-263145,Uttarakhand, India*

[6]*AICRP on Small Millets, GKVK, Bangalore-560065, Karnataka, India*

[*]*Email: laxmirawat1401@gmail.com*

Barnyard millet (*Echinochloa* spp.) is one of the oldest domesticated small millets in the semi-arid tropics of Asia and Africa (Sood *et al.,* 2020). The potential of this important small millet crop in addressing food and nutritional insecurities has been well recognized (Joshi *et al.,* 2020). However, compared to the efforts invested to improving major cereals, barnyard millet has received little research attention. The productivity of barnyard millet is reduced by a number of biotic constraints, among which grain smut caused by *Ustilago panici frumentacei* Bref. is potentially the most destructive and reported to cause nearly 60 % losses in grain yield (Jain *et al.,* 1997).

The presently grown cultivars of Indian barnyard millet are reported to have moderately resistant to susceptible and highly susceptible reaction against grain smut in multi- location trials. However, strong resistance against grain smut is reported neither in commercial cultivars nor in germplasm collections (Sood *et al.,* 2015). PRB 903 belonging to *E. esculenta* (Japanese type) is indigenously developed pure line selection from PRB 401 is an early maturing (mean duration 80 days) genetic stock with semi compact and purple colour inflorescence. It was observed to be highly resistant compared to the national checks and high yielding cultivars (VL 172, VL 207 and DHBM 93-3) in All India Coordinated Research Trials on Small Millets conducted over 22 locations in 6 years (Table 1).

The mean percent disease incidence over the years and locations (Table 1) due to grain smut in PRB 903 was very low (0.38%) and fell in highly resistant (HR) disease scale category compared to the national checks VL 172 (12.67%), VL 207 (11.70%) and DHBM 93-3 (5.72%). In disease screening nurseries of barnyard millet, resistant check is yet not available for grain smut. Therefore, the proposed genetic stock PRB 903 was used as a resistant check since 2018 to 2020 in national disease screening nurseries of barnyard millet. Interestingly, PRB 903 was reported to be

Table 1: Mean reaction of PRB 903 to grain smut disease (%) in coordinated trials

Item	*Locations*	*PRB 903 (PGS)*	*VL 172 (C1)*	*VL 207 (C2)*	*DHBM93-3 (C3)*
Mean of year 2010	4	0.00	15.44	12.55	-
Mean of year 2011	4	0.00	10.48	11.45	-
Mean of year 2012	4	0.00	12.10	24.40	-
Mean of year 2018	3	0.86	-	7.78	6.12
Mean of year 2019	4	0.52	-	7.05	4.39
Mean of year 2020	3	0.90	-	7.00	6.34
Mean over the years	-	0.38	12.67	11.70	5.72

PGS: Proposed Genetic stock; C1: Check 1; C2: Check 2; C3: Check 3

highly resistant by maintaining first rank among all the tested genotypes at different grain smut hot spot locations. Availability of highly resistant genetic stocks with minor grain smut incidence in different origin groups provides opportunity to significantly enhance the resistant level in commercial cultivars through hybridization and selection.

References

Jain AK, SK Jain and HS Yadava (1997). Assessment of yield losses due to grain smut in barnyard millet. *Indian Phytopathol,* **50(1):** 49-52.

Joshi DC, RP Meena and R Chandora (2020). Genetic Resources: collection, characterization, conservation and documentation. In: Singh M and Sood S (eds.) *Millets and Pseudocereals, Woodhead publishing*, pp 25-31.

Sood S, DC Joshi and A Pattanayak A (2020). Breeding Advancements in Barnyard Millet. In: Gosal S and Wani S. (eds.) *Accelerated Plant Breeding*, Vol 1. Springer Nature Switzerland, pp 391-409.

Sood S, RK Khulbe, AK Gupta, PK Agrawal, HD Upadhyaya and JC Bhatt (2015). Barnyard millet- a potential food and feed crop of future. *Plant Breed.* **134**: 135-147.

17. VL 386 (IC640692; INGR21128), a finger millet (*Eleusine coracana* L. Gaertn.) germplasm with resistance to foot rot, leaf blast, neck blast, finger blast, high harvest index and high grain yield

Arun Gupta[1], Salej Sood[2], RK Khulbe[3], Dinesh C Joshi[3*] and Chandrashekhara C[4]

[1]*ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana-132001, India*

[2]*ICAR-Central Potato Research Institute, Shimla-171001, Himachal Pradesh, India*

[3]*ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora-263601, Uttarakhand, India (developing Institute)*

[4]*ICAR-Indian Institute of Horticultural Research, Bengaluru-560089, Karnataka, India*

[*]*Email: Dinesh.Joshi@icar.gov.in*

Finger millet is an annual self-pollinated crop grown in Asia and Africa. Depending upon the severity, blast disease can cause yield loss to the tune of 50–90% whereas foot rot causes

considerable losses to the crop (Rao 2020). Blast affects finger millet at all stages of growth whereas symptoms of foot rot could be noticed 25-30 days after transplanting. Most of the landraces and a number of varieties are susceptible to highly susceptible to both the diseases (Sood *et al.*, 2019). Till date no genotype is reported in finger millet conferring resistance to both blast (leaf, finger and neck blast) and foot rot.

VL 386 has been indigenously developed from a cross between GE 440 **(**Early maturing core collection germplasm line belonging to Andhra Pradesh**)** and VL *Ragi* 149 (Blast resistant, High yielding old national variety). It was found resistant to foot rot (6.04%) in all Indian coordinated trials conducted during *kharif* 2015-2017 (Table 1). Likewise, the grade for leaf blast (2.5) and mean percent damage for finger blast (6.49%) and neck blast (8.02%) in coordinated trials (2015-2017) fell in the resistant category for VL 386 (Table 1). In addition to multiple disease resistance, VL 386 is reported to have high harvest index (31.75%) compared to both the national checks VL 352 (30.33%) and GPU 45 (28.52%). Based on the yield evaluation trials conducted over ten locations from 2015-2017, VL 386 reported to have 9.58% superiority in grain yield compared to the best check (VL 352). Identification of a genetic stock conferring resistance to the severe diseases and possessing desirable agronomic traits in the common genetic background will be highly useful in breeding for durable resistance and high yield in finger millet.

Table 1: Mean reaction of VL 386 against foot rot, leaf blast, finger blast and neck blast

Disease	*Year*	*Locations*	*VL 386*	*GPU 45 (C_1)*	*VL 352 (C_2)*	*GPU 67 (C3)*	*VR 708 (C4)*	*PR 202 (C5)*
Foot rot (%)	2015	2	2.2	0.0	0.7	1.0	1.7	0.0
	2016	4	7	7	9	-	-	-
	2017	2	8.93	13.3	6.68	8.63	-	10.30
	Mean		6.04	6.76	5.46	4.81	1.7	5.15
Leaf blast (G)	2015	9	1.5	1.3	2.0	2.1	2.5	2.6
	2016	11	3	4	3	-	4	-
	2017	3	2.8	3.1	3.3	4.0	-	4.22
	Mean		2.9	3.0	3.2			
Neck blast (%)	2015 (9)	9	6.70	3.80	9.40	10.7	16.0	10.6
	2016	11	11	5	14	-	21	-
	2017	3	6.36	6.86	10.33	8.93	-	7.43
	Mean		8.02	5.22	11.25	9.82	18.50	9.02
Finger blast (%)	2015	9	6.60	3.70	5.80	10.7	16.6	14.0
	2016	11	10	6	10	-	22	-
	2017	3	2.89	3.2	3.2	4.00	-	4.22
	Mean	-	6.49	4.30	6.33	7.35	19.30	9.11

References

Rao A (1990). Estimates of losses in finger millet (*Eleusine coracana*) due to blast disease (*Pyricularia grisea*). *Mysore J Agric Sci,* **24:** 57–60.

Sood S, Joshi DC and Chandra AK. (2019). Phenomics and genomics of finger millet: current status and future prospects. *Planta,* **250:** 731–751.

18. VL 399 (IC640693; INGR21129), a finger millet (*Eleusine coracana* L. Gaertn.) germplasm with broad resistance to finger blast and neck blast.

Arun Gupta[1], Salej Sood[2], RK Khulbe[3], Dinesh C Joshi[3*] and Rajashekhara H[4]

[1]*ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana-132001, India*

[2]*ICAR-Central Potato Research Institute, Shimla-171001, Himachal Pradesh, India*

[3]*ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora-263601, Uttarakhand, India (developing Institute)*

[4]*ICAR-Directorate of Cashew Research, Puttur, Dakshina Kannada -574202, Karnataka, India*

[*]*Email: Dinesh.Joshi@icar.gov.in*

Finger millet (*Eleusine coracana* L. Gaertn.) is a crop of antiquity with great historical, cultural and nutritional importance, particularly in Asia and Africa (Sood *et al.*, 2019). Among the various biotic constraints, blast is the major disease, which severely affects the finger millet production. Similar to rice, finger millet blast caused by *P. grisea* (Cke.) Sacc. is both economically significant and very destructive, causing over 50% yield loss (Joshi *et al.*, 2021). The use of blast-resistant finger millet varieties is an effective blast control strategy in the crop as it is compatible with low-cost input requirements of small-scale farmers who are the main growers of finger millet. Blast affects finger millet at all stages of growth and most of the landraces and a number of varieties are highly susceptible.

The proposed genetic stock VL 399 is indigenously developed from a cross between GPU 28 (High yielding, blast resistant late maturing cultivar) and VL 324 (high yielding, medium maturing locally adapted cultivar). It has mean maturity duration of 116 days in coordinated trials. In the coordinated trials conducted across 9 diverse locations, the percent damage due to finger blast in VL 399 was very low (7.90%) compared to the susceptible check *Uduru Malige* (29.09%) and fell in the same disease scale category (resistant reaction) of 5-10% with the resistant check GE 4449 (6.75%) (Table 1). Likewise, the percent damage due to neck blast in VL 399 was very low (9.9%) compared to the susceptible check *Uduru Malige* (37.47%) and fell in the same disease scale category (resistant reaction) of 5-10%

Table 1: Reaction of VL 399 against finger and neck blast

Disease	*Year*	*Locations*	*Percent damage*		
			VL 399	GE449 (RC)	Uduru Malige (SC)
Finger blast	2019	9	7.90	6.75	29.09
Neck blast	2019	8	9.93	8.36	37.47

*RC: Resistant check; SC: Susceptible check

with the resistant check GE 4449 (8.36%) (Table 1). Overall, VL 399 has broad resistance and had 2nd and 3rd rank for finger and neck blast resistance, respectively at all India level. Identification of a genetic stock conferring broad resistance to the severe diseases like neck and finger blast will be highly useful in breeding for durable resistance and high yield in finger millet.

References

Joshi DC, Meena RP and Chandora R (2021). Genetic Resources: collection, characterization, conservation and documentation. In: Singh M and Sood S (Eds) millets and Pseudocereals, Woodhead publishing, pp 25-31.

Sood S, Joshi DC, Chandra AK. *et al.* (2019). Phenomics and genomics of finger millet: current status and future prospects. *Planta* **250:** 731–751.

19. AKGMR 118 (IC640695; INGR21130), a sorghum (*Sorghum bicolor* L.) germplasm with resistance to grain mold, thresh grade

RB Ghorade[1], AR Gulhane[1], VV Kalpande[1], SB Thawari[1], IK Das[2], C Aruna[1], VU Sonalkar[1], GV Thakare[1], AR Bhuyar[1] and PS Kamble[1]

[1]*Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola-444001, Maharashtra, India*

[2]*ICAR-Indian Institute of Millets Research, Rajendranagar, Hyderabad-500030, Telangana, India*

Email: akola@millets.res.in

Sorghum (*Sorghum bicolor*) is the fourth most important cereal following rice, wheat and maize and staple food in the semi-arid parts of the world. Grain mold is the major biotic constrain in the way of production, marketing and utilization of kharif grain sorghum. It is one of the most important diseased of sorghum in many countries in Asia, Africa, North America, South America. The term Grain Mold Disease Complex (GDMC) has been used in few instances to describe this disease conditions (Prom *et al.* 2003). The disease is particularly important on improved, short- and medium-duration sorghum cultivars that mature during the rainy season in humid, tropical and subtropical climates. Several fungal species of the genera *Fusarium, Curvularia, Alternaria, Phoma, Bipolaris* and *Colletotrichum* have been reported to be associated with grain mold (Audilakshmi *et al.* 1999). *Curvularia lunata* and *Fusarium monoliforme* secrete amylase, cellulose and pectinases resulting in the disintegration of endosperm and germ tissues. These fungi also interfere with carbohydrate translocation to developing kernels causing reduction in size and weight of the kernels and ultimately germination of these grains. Therefore, identification of the resistant sources of grain mold in kharif sorghum is if prime importance. Considering this, a multilocation and multiseason grain mold screening trial entitled "National Grain Mold Nursery (NGN)" was formulated at national level by Indian Institute of Millets Research (IIMR), Hyderabad.

A national level trial was formulated by Indian Institute of Millets Research (IIMR), Hyderabad consisting of the entries contributed by the all the major centers of the AICSIP (All India Coordinated Sorghum Improvement Project) and conducted during the three years of 2016, 2017 and 2018 at the hot spot centers for grain mold i.e. Akola, Parbhani and Dharwad. In this connection, massive breeding programme for grain mold resistance has been continued at AICSIP Akola center and by utilizing the resistance sources identified in the programme, grain mold resistant lines have been developed at Akola center. AKGMR 118 contributed by Sorghum Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Maharashta) was tested for three years in the "National Grain Mold Nursery (NGN)" trial formulated at national level by Indian Institute of Millets Research (IIMR), Hyderabad. Trial consisted of the testing entries along with one resistant check for grain mold (B 58586) in loose panicle background and one susceptible check (Bulky yellow). The experiments were sown in randomized block design with three replications.

For the character grain mold field grade the resistant check B 58586 recorded the lowest grain mold infestation of 2.29, while the susceptible check exhibited the highest grain mold field grade of 6.66. The best promising entry was AKGMR 118 (3.50) followed by AKGMR 119 (3.65). For the character grain mold threshed grade the resistant check B 58586 recorded the lowest grain mold infestation of 1.83, while the susceptible check exhibited the highest grain mold field grade of 6.83. The best promising entry was AKGMR118 with the grain mold threshed grade of 3.45 followed by AKGMR 119 (4.08).

The promising genotype AKGMR 118 and the resistant check B 58586 exhibited resistant reaction towards both

field grade mold rating and threshed grain mold rating. This resistant reaction can be attributed to the lower percentage of both the fungi i.e. *Fusarium* and *Curvularia*.

Thus it was concluded form the present study that considering the resistant reaction as confirmed by AICSIP three years report against the grain mold disease, the promising genotype AKGMR 118 showing resistant reaction need to be exploited in breeding for grain mold resistance in sorghum as the donor parent in the crossing programme.

References

Audilakshami S, JW Stenhouse, TP Reddy and MVR Prasad (1999). Grain mold resistance and associated characters of sorghum genotype. *Euphytica*, **107:**91-103.

Prom L.K., T. Isakeit, R.Perumal, J.E. Erpelding, W.L.Rooney and C.W. Magill (2011). Evaluation of the Ugandan sorghum accessions for grain mold and anthracnose resistance. *Crop Protection,* **30:** 566-571.

20. VS 25 (IC640696; INGR21131), a little millet (*Panicum sumatrense* L.) germplasm with early flowering and early maturity

TSSK Patro[1*], N Anuradha[1], M Elangovan[2] and KB Palanna[3]

[1]*Agricultural Research Station, Vizinaagaram-535001, Andhra Pradesh, India.*

[2]*ICAR-Indian Institute of Millets Research, Hyderabad-500030, Telangana, India.*

[3]*University of Agricultural Sciences, GKVK, Bangalore-560065, Karnataka, India.*

[*]*Email: ars.vzm@gmail.com*

Little millet (*Panicum sumatrense* (L.)) is one among the seven small millets which is gaining popularity in modern days because of its triple security for food, fodder and nutrition. Early maturity in little millets is an important criterion to fit into double or triple cropping system and also to serve as contingent crop. Little millet genotype, VS 25 is a promising line for early flowering and early maturity. It is a pure line selection obtained from the germplasm collected from Gummalakshmipuram (GMPLSM 9), Andhra Pradesh.

The entry, VS 25 along with other test entries were tested at 6 locations under field conditions in states of Chhattisgarh, Jharkhand, Madhya Pradesh, Maharashtra and Orissa during 2019. VS 25 recorded earliness with respect to days to 50 % flowering (47 days) and maturity (71 days). Moreover, VS 25 has recorded significantly higher grain yield (1110 kg/ha) compared to check entries, DHLM 36-3(865 kg/ha), OLM 203(863 kg/ha) and BL 6(980 kg/ha). It was tested in station trials during 2017 to 2019 and in Coordinated trials during 2019 to 2020. It belongs to early maturity with medium plant height and medium number of productive tillers/plant. It is high yielding compared to late duration checks, DHLM 36-3, OLM 203 and BL 6 for grain yield. Little millet genotype, VS 25 is unique in terms of higher grain yield with earliness and hence can be used directly as a source for development of earliness with high yielding variety.

21. VR 1070 (IC640697; INGR21132), a finger Millet (*Eleusine coracana* L. Gaertn.) germplasm with resistance to neck blast and finger blast

TSSK Patro[1*], N Anuradha[1], M Elangovan[2], KB Palanna[3] and IK Das[2]

[1]*Agricultural Research Station, Vizinaagaram-535001, Andhra Pradesh, India*

[2]*ICAR-Indian Institute of Millets Research, Hyderabad-500030, Telangana, India*

[3]*University of Agricultural Sciences, GKVK, Bangalore-560065, Karnataka, India*

[*]*Email: ars.vzm@gmail.com*

Finger millet is one of the drought resistant small millet valued for its nutrient content in grains. It is a crop of both food and nutritional security. Blast is a major problem in finger millet crop which can hamper the production up to 70% in severe cases. Hence, finger millet improvement is oriented towards development of blast resistant high yielding varieties. Finger millet, VR1070 is finger and neck blast resistant line developed from pure line selection of IE No.2043. It belongs to medium maturity with medium plant height and medium number of productive tillers/plant.

The entry, VR 1070 recorded 16.2% less incidence of neck blast and 27.8% less incidence of finger blast compared to resistant check, Sri Chaitanya (VR 847) and 96.9% less incidence of neck blast and 97.3% less incidence of finger blast compared to susceptible check Champavathi (VR 708). The proposed entry along with other test entries and two checks were tested under high disease pressure under field conditions for consecutive five years from 2014 to 2018. It recorded less incidence of Finger blast (2.5%) among all the 3000 entries tested for finger blast resistance and also showed resistance to neck blast (3.5% Pooled data). Hence, VR 1070 is unique in terms of finger and neck blast resistance and hence can be used directly as a source for development of high yielding, finger and neck blast resistant varieties.

22. VR 1087 (IC640698; INGR21133), a (*Eleusine coracana* L. Gaertn.) germplasm with resistance to neck blast and finger blast.

TSSK Patro[1*], N Anuradha[1], M Elangovan[2], KB Palanna[3] and IK Das[2]

[1]*Agricultural Research Station, Vizinaagaram-535001, Andhra Pradesh, India*

[2]*ICAR-Indian Institute of Millets Research, Hyderabad-500030, Telangana, India*

[3]*University of Agricultural Sciences, GKVK, Bangalore-560065, Karnataka, India*

[*]*Email: ars.vzm@gmail.com*

Finger millet is one of the climate resilient small millet which serves both food and nutritional security. Blast is a major problem in finger millet crop which can hamper the production upto 70% in severe cases. Hence, finger millet improvement is oriented towards development of blast resistant high yielding varieties. Finger millet, VR 1087 is neck and finger blast resistant line developed by crossing VL 330 x GE 532. The cross was performed in 2006 for developing blast resistant high yielding variety and subsequent selections were made through pedigree method from F_2 to F_6. It was promoted at F_7 stage to Preliminary Yield Trial in 2013. It belongs to medium maturity with medium plant height medium number of productive tillers/plant. It is comparable with the check, Sri Chaitanya (VR 847) for grain yield.

The entry, VR 1087 recorded 3.2% less incidence of neck blast and 34.8% less incidence of finger blast compared to resistant check, Sri Chaitanya and 96.2% less incidence of neck blast and 97.5% less incidence of finger blast compared to susceptible check, Champavathi. VR 1081 along with other test entries and two checks were tested under high disease pressure under field conditions at All India Coordinated Research Project on Small Millets, Vizianagaram for consecutive five years from 2014 to 2018. It recorded 2.6% incidence of Finger blast among all the 3000 entries tested for finger blast resistance and also showed resistance to neck blast (3.8 % Pooled data). Hence, VR 1087 is unique in terms of neck and finger blast resistance and moreover it is a breeding line and hence it can be directly used as a source for development of high yielding, neck and finger blast resistant varieties.

23. SPV2591 (IC640645; INGR21134), a sorghum (*Sorghum bicolor* L. Moench.) germplasm with high total soluble sugar

Pummy Kumari[*1], DS Phogat[1], SK Pahuja[1], Satyawan Arya[1], Neeraj Kharor[1], Venkatesh Bhatt[2] and Vilas A Tonapi[2]

[1]*CCS Haryana Agricultural University, Hisar-125004, Haryana, India*

[2]*ICAR-Indian Institute of Millets Research, Hyderabad-500030, Telangana, India*

[*]*Emai1: pummy.hau@gmail.com*

In any agriculture based economy livestock plays important role in agriculture development. Fodder demand and supply gap is increasing day by day in the country and to bridge this gap there is essential requirement of good quality and insect pest and foliar disease resistant germplasm stock which can be used in breeding programme to develop improved varieties/hybrids. Forage sorghum [*Sorghum bicolor* (L.) Moench] is an important fodder crop of India and mainly grown during summer and *kharif* seasons in the states of western UP, Haryana, Punjab, Rajasthan and Delhi due to its greater adaptability, high fodder yield, better palatability. Which fulfills over two third of the fodder demand during summer and *kharif* season. But in addition to quantity, quality of feed also is a matter of concern because good quality feed provides better nutrition to the livestock and intern also affect quality of byproducts. Sorghum feed quality is mainly determined by TSS%, CP% and IVDMD%. There are some morphological markers which indicate sorghum feed quality like green and brown midrib, juiciness etc.

Major breeding objectives for forage sorghum improvement include its quality and resistance to insect pest and foliar diseases. This genotype SH1514/SPV2591 genotype is genotype is tall having 9.14% TSS and is also resistant to stem borer, shoot fly, anthracnose, zonate leaf spots, leaf blight, grey leaf spot. It is developed by pedigree breeding and tested in AICRP Trials, AVHT SC Trial *Kharif* 2019. In addition to this genotype is having crude protein content & IVDMD% statistically on par with checks and plant height more than checks.

Acknowledgement

Help and cooperation received from Dr. Vilas Tonapi, Director IIMR and his team working in IIMR, Hyderabad is thankfully acknowledged. We also thank Dr. M Elangovan who has helped in preparation of document. Cooperation and support received from HOD, Genetics and Plant Breeding and Director of Research, CCS HAU, Hisar is also duly acknowledged.

24. SPV2671 (IC640646; INGR21135), a sorghum (*Sorghum bicolor* L. Moench.) germplasm with resistance to anthracnose.

Pummy Kumari[1*], DS Phogat[1], SK Pahuja[1], Satyawan Arya[1], Dalvinder Pal Singh[1], Venkatesh Bhatt[2] and Vilas A Tonapi[2]

[1]*CCS Haryana Agricultural University, Hisar-125004, Haryana, India.*

[2]*ICAR-Indian Institute of Millets Research, Hyderabad-500030, Telangana, India.*

[*]*Emai1: pummy.hau@gmail.com*

In any agriculture based economy livestock plays important role in agriculture development. Fodder demand and supply gap is increasing day by day in the country and to bridge this gap there is essential requirement of good quality and insect pest and foliar disease resistant germplasm stock which can be used in breeding programme to develop improved varieties/hybrids. Forage sorghum [*Sorghum bicolor* (L.) Moench] is an important fodder crop of India and mainly grown during summer and *kharif* seasons in the states of western UP, Haryana, Punjab, Rajasthan and Delhi due to its greater adaptability, high fodder yield, better palatability. Which fulfills over two third of the fodder demand during summer and *kharif* season. But in addition to quantity, quality of feed also is a matter of concern because good quality feed provides better nutrition to the livestock and intern also affect quality of byproducts. Sorghum feed quality is mainly determined by TSS%, CP% and IVDMD%. There are some morphological markers which indicate sorghum feed quality like green and brown midrib, juiciness *etc*.

Major breeding objectives for forage sorghum improvement include its quality and resistance to insect pest and foliar diseases. This genotype SH1488/SPV2671 genotype is having high TSS% and low HCN and is also statistically on par for shoot fly and anthracnose resistance. It is developed by pedigree breeding and tested in AICRP Trials, IAVHT MC Trial *Khari*f 2019. In addition to this genotype is juicy, having good crude protein content & IVDMD% statistically on par with checks and leaf breadth more than checks.

Acknowledgement

Help and cooperation received from Dr. Vilas Tonapi, Director IIMR and his team working in IIMR, Hyderabad is thankfully acknowledged. We also thank Dr. M Elangovan who has helped in preparation of document. Cooperation and support received from HOD, Genetics and Plant Breeding and Director of Research, CCS HAU, Hisar is also duly acknowledged.

25. IC360831 (IC0360831; INGR21136), a French bean (*Phaseolus vulgaris* L.) germplasm with resistance to BCMV disease

Basavaraja T[1*], Manjunatha L[1], Mohar Singh[2], Rahul Chandora[2], Shiv Sewak[1] and NP Singh[1]

[1]*ICAR-Indian Institute of Pulses Research, Kanpur-208024, Uttar Pradesh, India*

[2]*ICAR-NBPGR Regional station, Shimla- 171004, Himachal Pradesh, India*

[*]*Email: Basavaraja.T@icar.gov.in*

Introduction

Rajmash/Common bean (*Phaseolus vulgaris* L.; $2n = 2x = 22$) is also known by kidney bean, French bean, Snap bean and dry bean etc. It is one of the most vital and highly relished pulse crop used for direct human consumption globally and is an important component of subsistence agriculture (Broughton *et al.,* 2003). Therefore, rajmash is regarded as "Grain of hope". In India, rajmash mainly produced by resource-poor farmers, small and marginal land holding farmers in the traditional production system that include rotation with vegetables and intercropping of climbing/ pole type varieties with grain amaranth, potato and maize during *Kharif* season in North Eastern Hilly (NEH) region. While, it grown as a sole crop by using bush types varieties during *rabi* season in northern plain and central India. It is growing under diverse geographical location in which crop may expose to varied climatic condition. However, crop was prone to many diseases, among them Bean common mosaic virus (BCMV) is one of the most destructive diseases of common bean, it is mainly transmitted by vector Aphid and having seed borne nature. The virus belongs to genus *Potyvirus* of family *Potyviridae* and in India, BCMV is of regular recurrence in North- western Himalayas with disease incidence ranging from 0.5 to 77.0 % (Sharma *et al.* 1998; Kapil *et al.* (2011)). Thus, the prevalence of BCMV disease has posed a serious threat for dry bean production in rajmash growing areas of Himachal Pradesh, Jammu & Kashmir, Sikkim, Uttar Pradesh and Uttarkhand. Hence, Identification of stable BCMV resistant donors is crucial in Rajmash/Common bean improvement programme.

Pedigree

During *rabi* 2015-16, 2016-17, 2017-18 and 2018-19 a set of 300 germplasm accessions were evaluated for agronomic traits and BCMV disease screening under natural epiphytotic

condition at IIPR, Kanpur by using BCMV resistant checks as Arun & Amber while, susceptible check as Jawala, Uday and IC341339. Disease scoring was done at three stages of crop growth *viz*., 45 DAS, 65 DAS and 90 DAS (Pathologist assistance involved). In these experiments, both agronomic and morphological data were recorded and based on BCMV percent disease incidence (PDI) data revealed that germplasm accession *viz.,* IC340947 and IC360831 were highly resistant against BCMV disease as they recorded 0.00% PDI over four consecutive seasons and *Kharif* rajmash is a highly remunerative pulse crop in hilly tract of Himachal Pradesh but BCMV disease is a major obstacle for grain production. During *Kharif* 2017 and 2018, a set of selected promising germplasm accession were screened against BCMV disease under natural field condition at NBPGR, Regional station, Shimla by using BCMV resistant checks as Arun & Amber while, susceptible check as Jawala and Uday. Based on agronomic traits and BCMV disease reaction scale the germplasm accession *viz.,* IC340947 and IC360831 were superior to check varieties and highly resistant against BCMV disease as they recorded 0.00% PDI. In addition to that, during *rabi* 2019-20 a set of selected promising germplasm accessions were screened against BCMV disease both under field and lab condition through sap inoculation method, similarly above experiments both agronomic and BCMV disease reaction was recorded at three different stages of crop growth. Likewise, previous experiments the germplasm accessions such as IC340947 and IC360831 were highly consistent and noticed highly resistant aganist BCMV disease and stable across the location and seasons. Thus, these donors being already utilized in disease breeding programme of Common bean. The germplasm identification committee under the chairmanship of Director, ICAR-IIPR visited the field on 09.03.2020 and examined the BCMV resistant donor in all aspects and GIC committee recommended for submission of germplasm registration proposal for unique germplasm identification.

Morpho-agronomic Characteristics

IC360831: It is having very good plant vigour at early plant stages of crop growth and It has adventitious root system, plant growth habit is pole (semi indeterminate) type, green stem pigmentation, the green leaves grow alternately on the stems, days to 50% flowering attaining at 70 days, flower colour bears light pink, leaflet shape is ovate, pod colour is pale green and pod shape is straight, pod length is 12.29 cm and physiological maturity attaining at 125 days and it produces yield about 20 quintals /ha.

Associated characters and cultivated practices

Rajmash grows well in areas where medium rainfall occurs, but not suited to the humid condition. Soil pH should be in the range of 5.5 - 6.0 to obtain maximum yield. The rajmash+ potato intercropping found quite profitable and efficient in irrigated areas. In hills, it is grown as a *Kharif* crop where as in plain grown as *rabi* crop. Seed rate varies with seed size. Bold seeded varieties with a test weight of 350-450 g need 120-140 kg seed/ha, while in small seeded varieties, it varies from 70-100 kg/ha and spacing for *Kharif* (Hills) - 45-50 cm x 8-10 cm while, Rabi & Spring - 40 cm x 10 cm (irrigated); 30 cm x 10 cm (Rain fed). For enhanced productivity, application of 90-120 kg N ha-1 has been found optimum. Half of the nitrogen should be applied as basal during sowing and rest half as top dressing after first irrigation and application of 60-80 kg P_2O_5/ha will give better yield. It requires 2 to 3 irrigations in NEPZ, Irrigation at 25 days after sowing is most critical followed by irrigation at 75 days after sowing. The crop matures in 125-130 days. A well-managed crop can easily give 20-25qtls/ha yields.

References

Sharma OP, Sharma PN and Sharma PK (1998). Comparative occurrence and distribution pattern of French bean mosaic in Himachal Pradesh. *Himachal J Agric Res*, **24:**165–171.

Kapil R, Sharma P, Sharma SK, Sharma OP, Sharma OP, Dhar JB and Sharma PN (2011). Pathogenic and molecular variability in Bean common mosaic virus infecting common bean in India. *Arch Phytopathol Plant Prot*, **44:**1081–1092.

Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P and J. Vanderleyden (2003). Bean (*Phaseolus* spp.)-model food legumes. *Plant Soil***, 252:** 55–128.

26. EC267301 (EC267301; INGR21137), a chickpea (*Cicer arietinum* L.) germplasm with ascochyta blight resistance

Gayacharan[1*], Upasana Rani[2], Sarvjeet Singh[2], Ashwani K Basandrai[3], Virender K Rathee[4], Kuldeep Tripathi[1], Neeta Singh[1], Girish P Dixit[5], JC Rana[6], Sushil Pandey[1], Ashok Kumar[1] and Kuldeep Singh[1]

[1]*ICAR-ICAR-National Bureau of Plant Genetic Resources, Pusa Campus-110012, New Delhi, India*

[2]*Punjab Agricultural University, Ludhiana-141004, Punjab, India*

[3]*Rice and Wheat Research Centre, CSKHPKV, Malan-176047, Himachal Pradesh, India*

[4]*Hill Agricultural Research and Extension Centre, CSKHPKV, Dhaulakuan-173031, Himachal Pradesh, India*

[5]*ICAR-Indian Institute of Pulses Research, Kanpur-208024, Uttar Pradesh, India*

[6]*Alliance of Bioversity International and CIAT Region-Asia, India Office, New Delhi-110012, India*

[*]*Email: gayacharan@icar.gov.in*

Introduction

EC267301 is resistant to Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labr. The pathogen *A. rabiei* is highly variable. Total 1970 chickpea accessions were screened at two hotspot locations viz. PAU, Ludhiana and HAREC, CSK HPKV, Dhaulakuan. The chickpea germplasm was screened and promising accessions were validated against the disease for over the six years under artificial epiphytotic conditions in two locations i.e. PAU, Ludhiana and CSK HPKV, Dhaulakuan. An artificial standard inoculation procedure was followed for uniform spread of the pathogen (Gayacharan *et al.*, 2020). The disease reaction: 0 (immune) to 9 (highly susceptible) is as given below:

Screening of EC267301 for Ascochyta blight resistantance

Genebank I.D.	Ludhiana			HAREC, CSK HPKV, Dhaulakuan
	2016-17	2018-19	2019-20	2015-16
EC267301	3	3	3	1
L550(susceptible check)	9	9	9	9
JG62 (susceptible check)	9	9	9	9

Morpho-agronomic Characteristics

Flower Color: White	Seed shape	Angular shape
Seed surface: Rough	Seed color	Beige
100 seed weight: 21.3		

References

Gayacharan, U Rani, S Singh, AK Basandrai, VK Rathee, K Tripathi, N Singh, GP Dixit, JC Rana, A Kumar and K Singh (2020). Identification of novel resistant sources for ascochyta blight (*Ascochyta rabiei*) in chickpea. *Plos one*, **15(10):** e0240589.

ICAR-NBPGR (2018) Annual Report 2017-2018, ICAR- National Bureau of Plant Genetic Resources, New Delhi, India, 181p. (Page no. 55)

ICAR-NBPGR (2019) Annual Report 2018-2019, ICAR- National Bureau of Plant Genetic Resources, New Delhi, India, 230p. (Page no. 71)

ICAR-NBPGR (2020) Annual Report 2019, ICAR- National Bureau of Plant Genetic Resources, New Delhi, India, 236 p. (Page no. 69)

27. IC248147 (IC248147; INGR21138), a chickpea (*Cicer arietinum* L.) germplasm with ascochyta blight resistance

Gayacharan[1*], Upasana Rani[2], Sarvjeet Singh[2], Ashwani K Basandrai[3], Virender K Rathee[4], Kuldeep Tripathi[1], Neeta Singh[1], Girish P Dixit[5], JC Rana[6], Sushil Pandey[1], Ashok Kumar[1] and Kuldeep Singh[1]

[1]*ICAR-ICAR-National Bureau of Plant Genetic Resources, Pusa Campus-110012, New Delhi, India*

[2]*Punjab Agricultural University, Ludhiana-141004, Punjab, India*

[3]*Rice and Wheat Research Centre, CSKHPKV, Malan-176047, Himachal Pradesh, India*

[4]*Hill Agricultural Research and Extension Centre, CSKHPKV, Dhaulakuan-173031, Himachal Pradesh, India*

[5]*ICAR-Indian Institute of Pulses Research, Kanpur-208024, Uttar Pradesh, India*

[6]*Alliance of Bioversity International and CIAT Region-Asia, India Office, New Delhi-110012, India*

[*]*Email: gayacharan@icar.gov.in*

Introduction

IC248147 is resistant to Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labr. The pathogen *A. rabiei* is highly variable. Total 1970 chickpea accessions were screened at two hotspot locations viz. PAU, Ludhiana and HAREC, CSK HPKV, Dhaulakuan. The chickpea germplasm was screened and promising accessions were validated against the disease for over the six years under artificial epiphytotic conditions in two locations i.e. PAU, Ludhiana and CSK HPKV, Dhaulakuan. An artificial standard inoculation procedure was followed for uniform spread of the pathogen (Gayacharan *et al.,* 2020). The disease reaction: 0 (immune) to 9 (highly susceptible) is as given below:

Screening of IC248147 for Ascochyta blight resistantance

Genebank I.D.	*PAU, Ludhiana*			*HAREC, CSK HPKV, Dhaulakuan*
	2017-18	*2018-19*	*2019-20*	*2014-15*
IC248147	2.5	3	3	5
L550 (susceptible check)	9	9	9	9
JG62 (susceptible check)	9	9	9	9

Morpho-agronomic Characteristics

Seed type	Desi	Days to 50% flowering	110 days
Flower Color	Pink	Seed shape	Angular
Seed Color	Brown beige	100 seed weight	12.5 g

References

Gayacharan, U Rani, S Singh, AK Basandrai, VK Rathee, K Tripathi, N Singh, GP Dixit, JC Rana, A Kumar and K Singh (2020). Identification of novel resistant sources for ascochyta blight (*Ascochyta rabiei*) in chickpea. *Plos one*, **15(10):** e0240589.

ICAR-NBPGR (2018) Annual Report 2017-2018, ICAR-National Bureau of Plant Genetic Resources, New Delhi, India, 181p. (Page no. 55)

ICAR-NBPGR (2020) Annual Report 2019, ICAR- National Bureau of Plant Genetic Resources, New Delhi, India, 236 p. (Page no. 69)

28. VRPSel-17 (No-17) (IC640701; INGR21139), a pea (*Pisum sativum* subsp. *hortense*) germplasm of vegetable pea genotypes bearing single flower per peduncle on all the floral nodes

Jyoti Devi[1*], RK Dubey[1], SK Sanwal[1,2], Subhas Chandra[1] and PM Singh[1]

[1]*ICAR-Indian Institute of Vegetable Research, Varanasi-221403, Uttar Pradesh, India*

[2]*ICAR- Central Soil Salinity Research Institute, Karnal-132001, Haryana, India*

[*]*E-mail: jyoti17iivr@gmail.com*

Introduction

The number of flower/peduncle (FPP) is an economic trait in peas having agricultural and evolutionary significance that is influenced by a variety of factors including the environment. Thus, a varied expression of FPP can be seen within a single genotype as well within single plant of a genotype with a definite pattern, such as a single flower at the lower few nodes followed by two or three flowers on some of middle nodes, that again terminates into single/double flower at the upper nodes. As a result, it is difficult to get single/double/multi- flower on all the flowering nodes of a plant or a genotype, that usually create confusion in genetic studies especially devoted for flowering traits in peas.

VRPSel-17 (No-17) is a mid-season genotype of vegetable pea, unique in the fact that it bears only one flower (white) on all its flowering nodes, and not affected by external growing conditions including the environment. The genotype is tall in growth habit with longer internodal length and peduncles.

Table 2: DUS characterization of 'VRPSel-17' as per the guidelines provided by Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) Government of India

Sl. No.	*Type of Assessment*	*DUS Characteristics*	*VRP Sel-17'*
1.	VS	Stem: Anthocyanin colouration	Absent
2.	VG	Foliage: colour	Green (Yellow green group 147A) *
3.	VG	Foliage: waxy bloom	Absent
4.	VG	Leaf: leaflets	Present
5.	VS	Leaf: axil colour	Green
6.	VG	Stipule: rabbit eared stipules	Present
7.	VG	Stipule: type	Normal
8.	VG	Flower: opening (days)	Medium (50.1)
9.	VG	Flower: standard petal colour	White
10.	VS	Pod: number/axil	Single
11.	VG	Pod: curvature	Absent
12.	VS	Pod: shape of distal part	Blunt
13.	VG	Pod: intensity of green colour	Light green (Yellow Green Group 146B) *
14.	MS	Plant: height	Long (162.7cm)
15.	VG	Seed: shape	Dimpled
16.	VG	Seed: surface	Smooth
17.	VG	Seed: cotyledon colour	Creamy (GYG161C)
18.	MG	Seed: Weight of 1000-seed (dry)	170g
19.	VG	Seed: testa mottling	Absent
20.	VG	Seed: parchment	Absent

MG: Measurement by a single observation of a group of plants or parts of plants
MS: Measurement of a number of individual plants or parts of plants
VG: Visual assessment by a single observation of a group of plants or parts of plants
VS: Visual assessment by observation of individual plants or parts of plants
* Intensity of color measured by Royal Horticultural Society Color Charts (1804)

Over the years, the genotype exhibited consistent flowering behaviour of single FPP on all the reproductive nodes . The genotype is kept as unique selection from the germplasm, maintained at ICAR-IIVR, Varanasi since last few years and presently in use for various genetic studies.

Morpho-agronomic characteristics

The genotype is characterized by its indeterminate growth habit (average plant height 162.7cm) with longer internodal (8.5cm) and peduncle length (6.2cm) that reflects as a dominate trait in its off-springs. Pods are 6-7 cm long, green in colour having 20-25 pods per plant with four to five seeds per pod. Seeds are smooth, yellowish cream (GYG161C) in colour having 100-Green seed weight of 37-40g while 1000 dry seed weight of 17g.

Associated characters and cultivated practices

The genotype VRPSel-17 is also resistant to powdery mildew and rust under natural field conditions. This genotype also exhibited highest total phenolics content (TPC) and total flavonoids content (TFC) of 37.80 mg GAE/100 g fw) and 15.61 (mg CE/100 g fw), respectively among various white flowered genotypes studied (Devi *et al.,* 2019). Despite its low pod yield (60-70g/plant), this genotype can be used in a variety of genetic studies, including inheritance of flower numbers, flowering time, peduncle traits, seed and pod characters, disease studies, and so on. The genotype is suitable for November sowing under north Indian conditions. The optimum temperature for seed germination is about 22 ± 2°C. Furthermore, standard cultivation practises can be adopted to raise a healthy garden-pea crop.

References

Devi J, Dubey RK, Mishra GP, Sagar V, Verma RK, Singh PM and Singh J (2021). Inheritance and stability studies of multi–flowering trait in vegetable pea (Pisum sativum L.), and its contribution in yield improvement. *Sci. Hortic.* 287: 110235. *doi.org/10.1016/j.scienta.2021.110235*.

Devi J, Sanwal SK, Koley TK, Mishra GP, Karmakar P, Singh PM and Singh B (2019). Variations in the total phenolics and antioxidant activities among garden pea

(*Pisum sativum* L.) genotypes differing for maturity duration, seed and flower traits and their association with the yield. *Sci. Hortic.*, **244:**141–150.

29. IPFD 18-14 (IC636671; INGR21140), a pea (*Pisum sativum* L.) germplasm with extra early flowering, early maturity and yellow cotyledon

AK Parihar*, GP Dixit, Anil Kumar Singh, Nitin Kumar and NP Singh

[1]*ICAR-Indian Institute of Pulses Research, Kanpur-208024, Uttar Pradesh, India*

**Email: ashoka.parihar@gmail.com*

Introduction

Field pea is acknowledged as a minor winter season pulse crop of India and is being cultivated in 0.82 mh area with 0.99 mt production. The major field pea growing states in the country are Uttar Pradesh, Madhya Pradesh, Bihar, Assam and Odisha (Anonymous, 2019). This crop is an admirable source of protein, starch, minerals and vitamins, as a result, it is widely used as an important component in many food industries worldwide (Parihar *et al.* 2016).The average productivity of field pea at global level is near 2.0 tonnes per hectare whereas in India it is quite low and being oscillated between 0.8-1.0 tonnes/ha (FAOSTAT, 2019). The major constraints in achieving potential yield in field pea are prevalence of biotic and abiotic stresses especially at terminal stage. Of them powdery mildew, rust and high temperature are the major concern for pea breeders (Parihar *et al.* 2020a). In the recent past, it has been noticed that early shoot up (mid of Jan-Feb) in temperature (>25°C) usually coincides with the flowering or pod filling stage of pea ultimately causing noteworthy reduction in productivity (Parihar *et al.* 2020b). In addition, prolonged kharif season compelled farmers to go for late planting which ultimately led to high temperature (> 30°C) exposure of pea crop during critical growth stages resulting into remarkable yield penalty. At the same time, the diseases pressure particularly of powdery mildew and rust remains high in late planted pea crop (Mishra *et al.* 2020). The central parts and rice fallow situation is a typical short window available which is always terminated by drought and heat stresses. In such situations, extra early maturing varieties (<90days) are the best options and may enhance monetary returns to farmers. In addition, the earliness is the ultimate trait to maximize the yields per unit time and per unit area in any cropping system by vacating the field for timely planting of succeeding crops (Dixit *et al.* 2014). Therefore, keeping in mind above facts, an urgent need was felt to develop extra early genotypes in field pea. Consequently, sincere breeding efforts were made at ICAR-IIPR, Kanpur using inter- specific hybridization to recover desirable segregants in filial generations. To develop extra early materials, a targeted inter-specific crosses were made between selected early type released varieties of Field pea (*Pisum sativum* L. var. *arvense*) and Garden pea (*Pisum sativum* L. var. *hortense*) since earliness trait is prominent in garden pea. The segregating generations were handled using pedigree methods and promising plants were selected in different generations. Based on the earliness, superior fixed lines were identified and of them most promising lines were evaluated consecutively for two years *viz*. 2017-18

and 2018-2019 in winter season for earliness with available early type checks *viz*., Arkel, VRP 6, AGETA 6, DDR 30, DDR 23 and VRP 22. In addition, staggered planting (04/11/18; 29/12/18; 12/01/19) of the same set was also done during winter 2018-19. Based on over the years and staggered planting performance, one line IPFD 18-14 (DDR 23 x VRP 22) was found extra early. This genotype had flowered and matured in less than 36 days and 75 days, respectively. Hence, this extra early fieldpea genotype can be used as donor to develop short duration varieties in fieldpea breeding program and also for various genetical studies.

Morpho-agronomic characteristics

The genotype (IPFD 18-14) is semi-dwarf type (50-60 cm) and leafy type. It has white colour flowers, white & round seed and yellow cotyledon. It flowered and matured in 30-35 days and 80-85 days respectively with 18.0-19.0 gm 100-seed weight. The average yield of the genotype is more than 1.0t/ha.

Associated characters and cultivated practices

This genotype also has powdery mildew resistance and rust tolerance. It can be grown on different type of soil; however, a well drained soil is essential to achieve good yield potential of this genotype. The field should be well prepared by two to three ploughings. The seeds treatment should be done with fungicide like Thirum/Captan/Carbendazim @ 3.0 g/kg seed + rhizobium culture @1 packet/10 kg + Trichoderma @ 4.0 g/kg at 4-5 days before sowing. The optimum sowing time is October 15-Novemebr 15 with 100 kg/ha seed rate. At the time of field preparation 40 kg N, 40-60 kg P_2O_5:20-30 kg K_2O: 20 kg S: 15 kg $ZnSO_4$ should be applied as basal application. First irrigation should be given at 45 days and second, if needed, at pod filling stage.

This genotype has good worth as a donor in future fieldpea breeding programme towards development of extra early fieldpea varieties and in other basic studies.

References

Anonymous (2019). Project coordinators report, (2018-19) All India Coordinated Research Project (AICRP) on MULLaRP, ICAR-IIPR, Kanpur.

Dixit GP, AK Parihar and S Gupta (2014). Perspectives for increasing fieldpea production in India. In: Handbook on minor and imported pulses of India 2014. Published by Commodity India.com.

FAO. 2019. Food and Agriculture Organization Statistics. http://www.fao.org/faostat/en/#data/QC Mishra RK, AK Parihar, AK Singh, M Mishra and GP Dixit (2020) Identification of resistant sources for rust and Powdery mildew in Field pea. In: (Dixit *et al.* 2020). Abstract: International conference on pulses as the climate smart crops: challenges and oppurtunities, ICAR-Indian Institute of Pulses Research, Kanpur, pp467

Parihar AK, A Bohra and GP Dixit (2016). Nutritional Benefits of Winter Pulses with Special Emphasis on Peas and Rajmash. In: Biofortifications of food crops, *Springer publication* (ed. Singh *et al* 2016), Pp 61-71, DOI 10.1007/978-81-322-2716-8_6

Parihar AK, GP Dixit, A Bohra, DS Gupta, AK Singh, N Kumar, D Singh and NP Singh (2020a). Genetic Advancement in Dry Pea (*Pisum sativum* L.): Retrospect and Prospect. In: SS Gosal and SH Wani (Eds): Accelerated Plant Breeding, Volume 3, Springer, *Springer Nature*, Switzerland, Pp283-341.

Parihar AK, AK Singh, A Lamichaney, GP Dixit and NP Singh (2020b) Effects of high temperature exposure on various yield attributes in Field pea (*Pisum sativum* L. In: (Dixit *et al.*2020). Abstract: International conference on pulses as the climate smart crops: challenges and opportunities, ICAR-Indian Institute of Pulses Research, Kanpur, pp46

30. IPC 2020-198 (IC640699; INGR21141), a chickpea (*Cicer arietinum* L.) germplasm with high (three) number of seeds per pod

Biswajit Mondal[1*], Yogesh Kumar[1], Avinash Kumar Srivastava[1], Uday Chand Jha[1], Revanappa Biradar[2], Archana Singh[3], Shiv Sewak[1], Sushil Kumar Chaturvedi[4], Girish Prasad Dixit[1], Khela Ram Soren and Narendra Pratap Singh[1]

[1]*ICAR-Indian Institute of Pulses Research, Kanpur-208024, Uttar Pradesh, India*

[2]*IIPR Regional Research Center, Dharwad-580005, Karnataka, India*

[3]*IIPR Regional Research Center, Bhopal-462001, Madhya Pradesh, India*

[4]*College of Agriculture, RLBCAU, Jhansi-284003, Uttar Pradesh, India*

[*]*Email: biswagpb@gmail.com*

Pedigree

IPC 20-198 = IPC 2006-88 (*Cicer arietunim*)/ILWC 179 (*Cicer echinospermum*) IPC 2006-88 (recipient parent) = IPC 95-1 /H 82-2

Trait for registration

3 seeds/ pod in ~30% of pods per plant

Plant breeding intervention

The parental lines (IPC 2006-88 and ILWC 179) have 1-2 seeds per pod. The cross was attempted to generate deliberate variability through wide hybridization. The F_2 was bulked and generation advancement till F_5 was carried out through single plant selections and growing progeny rows from them (Pedigree breeding). However, in the F_5 generation

Table 1: Performance of the potential RILs with pods having >2 seeds/pod and desirable seedcharacteristics up to the consumer preference

RIL no.	*Pods with 1 seed/ pod*	*Pods with 2 seeds/ pod*	*Pods with 3 seeds/ pod*	*Pods with 4 seeds/ pod*	*Total pods in 3 plants*	*% of pods with >3 seeds/pod*
62	28	56	14	5	103	19.41
63	18	24	17	3	62	32.25*
64	15	41	17	3	76	26.31
30	26	33	16	0	75	21.33
15	21	35	16	0	72	22.22
43	18	30	13	0	61	21.31

Advanced breeding line IPC 20-198 is the derivative of the line no. 63 in the table.

segregating lines, pods with 3 seeds were observed in high frequency in this population. The Singe Plant Selections (SPS, 137 no.s) were carried out in F_5 and F_6 progeny rows were raised. Data was generated for the number of seeds per pod in three plants in each row. Multidimensional visualization of the raw data was developed in the parallel coordinate plot (Wegman, 1991) and potential RILs were selected based on the target trait and desirable seed traits (angular seed shape, yellow testa colour and testa without tuberculation).

Population structure

Total F_6 population strength: 137, Plants with 1, 2 Seeds/ Pod: 3, Plants with 1, 2, 3 Seeds/Pod: 106 Plants with 1, 2, 3 & 4 Seeds/Pod: 28

References

Mondal B, Chaturvedi SK, Das A, Kumar Y, Yadav A, Shiv Sewak and Singh NP (2020). Embryo rescue and chromosomal manipulations. In: Chickpea: Crop Wild Relatives for Enhancing Genetic Gains, Mohar Singh (Ed.) Elsevier Academic Press pp 95-130.

Annual Report 2019. Indian Institute of Pulses Research, Kanpur, pp 15. (2019-20)

Annual Report 2018-19. Indian Institute of Pulses Research, Kanpur, pp 15.

31. ICC 12315 (IC640700; INGR21142), a chickpea (*Cicer arietinum* L.) germplasm with tolerance to post emergent herbicide Imazethapyr

Biswajit Mondal[1*], Yogesh Kumar[1], Chaitanya Prasad Nath[1], Avinash Kumar Srivastava[1], Uday Chand Jha[1], Revanappa Biradar[2], Archana Singh[3], Sushil Kumar Chaturvedi[4], Narendra Kumar[1], Shiv Sewak[1], Girish Prasad Dixit[1], Srinivasan Samineni[5], PM Gaur[5] and Narendra Pratap Singh[1]

[1]*ICAR-Indian Institute of Pulses Research, Kanpur-208024, Uttar Pradesh, India*

[2]*IIPR Regional Research Center, Dharwad-580005, Karnataka, India*

[3]*IIPR Regional Research Center, Bhopal-462001, Madhya Pradesh, India*

[4]*College of Agriculture, RLBCAU, Jhansi-284003, Uttar Pradesh, India*

[5]*International Crops Research Institute for the Semi-Arid Tropics, Hyderabad-502324, Telangana*

[*]*Email: biswagpb@gmail.com*

Pedigree

Selection from H76-49, collected from Haryana.

Trait for registration

Tolerance to post emergent herbicide Imazethapyr (1.5X @ 150g/ ha.) with > 85% of survival and seed setting.

Plant breeding intervention

Desi chickpea genotype ICC 12315 (germplasm accession/ breeding line) was received from ICRISAT under DAC funded project (Mechanical

harvesting and herbicide tolerance). The material was screened for resistance to post emergent herbicide Imazethapyr under supra optimal dose (2X @ 200g/ ha.) along with other genotypes. Heterogeneous response was observed in the treated population at maturity and Single Plant Selections (SPS) setting seeds were retrieved for screening in next season. For three consecutive seasons, spraying the herbicide and selection were carried out. ICC 12315 was observed to be possessing tolerance to post emergent herbicide Imazethapyr (1.5X @ 150g/ ha.) with > 85% of survival and seed setting over years.

Morphological characteristics

Under normal conditions (without herbicide treatment), ICC 12315 has a semi erect plant architecture with medium height (45-50 cm). It initiates flowering in 85-88 days after sowing and matures in 127-130 days. The flower colour is pink. Seeds have light brown testa colour with a hundred seed weight of 21.32 g. ICC 12315 when sprayed with Imazethapyr (1.5x@ 150g/ha.) at 30-40 says after sowing with the weed population competing with crop, it initiates

flowering at 90-93 days after sowing and matures in 127-130 days with the unsprayed crop with <5% yield penalty. The herbicide sprayed crop attends a height of 30-35 cm at maturity. The seeds of the sprayed crop have brown testa colour and a hundred seed weight 18-21 g.

References

Mondal B, Chaturvedi SK, Srivastava AK, Dixit GP, Kumar Y, Yadav RKS and Yadav A (2018). Post-emergence herbicide tolerance in chickpea: Progress and prospects. *Pulses Newsletter*, **29(4):** 4.

Annual Report 2018-19. Indian Institute of Pulses Research, Kanpur, pp 15.

Annual Report Year 3 (1 April 2015 - 31 March 2016): Developing Chickpea Cultivars suited to Mechanical Harvesting and Tolerant to Herbicides. pp. 47 http://nfsm.gov.in/Project/ICRISAT/2016-17/ICRISAT3ReportChickpeaJuly2016.pdf

32. IC251372 (IC251372; INGR21143), a Vigna (*Vigna glabrescens* Marechal & al.) germplasm with photo-thermo period insensitivity

Aditya Pratap[1*], Partha Sarathi Basu[1], Sanjeev Gupta[1], Latha Madhavan[2] and NP Singh[1]

[1]*ICAR-Indian Institute of Pulses Research, Kanpur-208024, Uttar Pradesh, India*

[2]*NBPGR Regional Station, Vellanikkara, Thrissur-680656, Kerala, India*

[*]*E-mail: adityapratapgarg@gmail.com*

The genus *Vigna* comprising >200 different species is highly variable with pantropic distribution. A number of the *Vigna* species *viz.*, mungbean [*V. radiata* (L.) Wilczek], blackgram [*V. mungo* (L.) Hepper], cowpea [*V. unguiculata* (L.) Walp], adzuki bean [*V. angularis* (Willd.) Ohwi & Ohashi], bambara groundnut [*V. subterranea* (L.) Verdn.], moth bean [*V. aconitifolia* (Jacq.)] and rice bean [*V. umbellata* (Thunb.) Ohwi & Ohashi] are widely cultivated and consumed and therefore, hold tremendous agronomic promise and economic importance. Among these, mungbean and blackgram are the most popular pulse crops of the *Vigna* group and India is their largest producer, consumer and importer in the world (Pratap *et al.* 2012). Both these crops can be grown across a wide range of adverse soil and climatic conditions, and seasons (spring, summer, winter and rainy seasons) in different parts of the country as a sole, relay or intercrop (Pratap *et al.* 2013). Therefore, these crops also offer an opportunity for their horizontal as well as vertical expansion in large areas. However, the biggest hindrance in horizontal expansion of blackgram and mungbean in India is their photo- and thermo-sensitive behaviour which necessitates breeding of an array of genotypes for different agro-climatic zones of the country. Photo–thermo period sensitivity makes these crops vulnerable to photoperiod and temperature fluctuations, thereby affecting their yield potential drastically, especially when the same variety of these crops is grown across different seasons. Therefore, there is a need to develop cultivars which are largely insensitive to photo- and thermo-period and can be grown over large areas across seasons and agro-climatic zones. However, breeding for photo- and thermo-insensitivity requires robust donors for this trait without a possibility of linkage drag. Keeping this in view, this study aimed at identification of photo- and thermo-insensitive donors in the *Vigna* species so that these could be utilized in mungbean and blackgram improvement programmes through introgression breeding.

A set of 56 accessions of *Vigna* belonging to 13 species (eight accessions of *V. trilobata*, seven of *V. umbellata*, five each of *V. mungo* and *V. hainiana*, four each of *V. sylvestris* and *V. vexillata*, three each of *V. radiata*, *V. sublobata, V. dalzelliana* and *V. pilosa*, and one each of *V. glabrescens, V. trinernia* and *V. unguiculata*) and eight cultigens (four each of mungbean and blackgram) was grown under natural field conditions in the tropical climatic conditions of Kanpur (26°28' N, 80°24'E) in two consecutive years (2011-13). All the individual plots of 56 accessions were observed every alternate day and the dates of important phenological events and characterization parameters as per NDUS guidelines (IIPR, 2010) were recorded. Viability of the fresh pollen samples in those accessions which survived through the rainy, summer and winter seasons was determined on the basis of observations on stainability of fresh pollen grains. On the basis of phenological events, physiological parameters as well as pollen viability studies, two accessions, *viz., V. umbellata* (IC251442) and *V. glabrescens* (IC251372) were identified to be largely photo- and thermo-period tolerant. Both these accessions behaved normally and were able to flower and set pods at extreme temperatures as high as 43.9°C and as low as 2.7°C. Pollen viability studies were also conducted which recorded viable pollen (>75% at 2.7°C and >85% at 41.9°C) and normal pollen tube growth at both the extremes of temperature. The identified *V. glabrescens* accession has long, constricted pods and dark green, mottled seeds while *V. umbellata* has smooth, curved pods and shining, oval, large seeds. Both these accessions have several other desirable characters besides photo- and thermo-period tolerance, such as long and bold pods, profuse branching and resistance to all major diseases of *Vigna* species, and therefore these could be potential donors for the above traits in *Vigna* through introgression breeding. Earlier *V. radiata* X *V. umbellata* and *V. mungo* X *V. umbellata* crosses

have been successfully generated to transfer resistance to MYMV and other desirable traits into mungbean (Singh *et al.* 2003; Chaisen *et al.* 2013) and urdbean (Pal *et al.*, 2005). Both these accessions are currently being utilized in developing photo–thermo tolerant genotypes in cultivated *Vigna* species.

References

IIPR (2010). National Test Guidelines for the conduct of tests for distinctness, uniformity and stability of Mungbean (*Vigna radiata* L.) and Urdbean (*Vigna mungo* L.), 1-25. All India Coordinated Research Project on MULLaRP, Indian Institute of Pulses Research, Kanpur, India.

Chaisan T, P Somta, P Srinives, S Chanprame, R Kaveeta and S Dumrongkittikule (2013). Development of tetraploid plants from an interspecific hybrid between mungbean (*Vigna radiata*) and rice bean (*Vigna umbellata*). *J. Crop Sci. Biotec,* **16:** 45—51.

Singh K P, PK Sareen, Monika Sareen and A Kumar (2003). Interspecific hybridization studies in Vigna radiata L. Wilczek and Vigna umbellata L. *Natl. J. Plant Improv,* 5**:** 16-18.

Pal SS, JS Sandhu and I Singh (2005). Exploitation of genetic variability in interspecific cross between *Vigna mungo* X *V. umbellata*. *Indian J. Pulses Res,* **18:** 9-11.

33. IC15925 (IC272450; INGR21144), a chickpea (*Cicer arietinum* L.) germplasm with heat tolerance

Uday Chand Jha[1*], Yogesh Kumar[1], Avinash K Srivastava[1], Biswajit Mondal[1], SK Chaturvedi[2], Archana Singh[3] and NP Singh[1]

[1]*ICAR-Indian Institute of Pulses Research, Kanpur-208024, Uttar Pradesh, India*

[2]*Rani Lakshmi Bai Central Agricultural University, Jhansi-284003, Uttar Pradesh, India*

[3]*ICAR-IIPR Regional Station, Phanda, Bhopal-462030, Madhya Pradesh, India*

[*]*Email: uday_gene@yahoo.co.in*

Heat stress remains one of the important abiotic stresses causing significant yield loss in various crops including chickpea across the globe (Jha *et al.* 2014). Thus, identifying heat tolerant genotype is becoming important for developing heat tolerant chickpea genotype. A set of 182 chickpea along with genotype ICC15925 was tested for four season under normal sown and late sown condition (during 2017-18 and 2018-2019 years) along with the check ICCV92944 (heat tolerant check) and ICC10685 (heat sensitive check). Based on various morphophysiological and yield parameters ICC15925 was identified to be heat tolerant (Jha *et al* 2021). This genotype matures in 125 days under normal condition and under late sown condition it matures in 108 days. The yield of this genotype was noted to be superior to the check (ICCV92944/JG14) under late sown condition during 2017-2018 (Annual report 2018). In the year 2018-2019, the yield of this genotype was noted to be superior to the check (ICCV92944/JG14) under late sown condition (Annual report 2019). It matured in 126 days under normal sown condition and 106 days under late sown condition. Additionally, characterisation of physiological traits *viz*., cell membrane stability, peroxidase, super oxidase mutase was done for this genotype. Thus, based on both physiological and yield parameters this genotype showed heat tolerance. Besides, this genotypes has been used as donor for transferring heat tolerant traits to elite chickpea cultivars *viz*., in JG11, Pusa362, JG315 through crossing programme (Annual report 2018-2019). Therefore, in future this genotype can be used further used as donor for transferring heat tolerance trait to other high yielding chickpea yet heat stress sensitive cultivar.

References

Annual report 2017-18, IIPR, Kanpur Pp2 Annual report 2018-19, IIPR, Kanpur Pp5

Jha UC, Bohra A, Singh NP (2014). Heat stress in crop plants: its nature, impacts and integrated breeding strategies to improve heat tolerance. *Plant Breed,* **133:** 679-701

Jha UC, Jha R, Thakro V, Kumar A, Gupta S, Nayyar H, Basu P, Parida SK and Singh NP (2021). Discerning molecular diversity and association mapping for phenological, physiological and yield traits under high temperature stress in chickpea (*Cicer arietinum* L.). *J. Genet.*, **100:** 1-5

34. IC635410; BG-114-1 (IC635410; INGR21145), a bottle gourd [*Lagenaria siceraria* (Mol.) Standl.)] germplasm with resistance to gummy stem blight and short cylindrical fruit character

Dhananjaya MV[*], Sandeep Kumar GM, Varalakshmi B and Mahesh B

ICAR-Indian Institute of Horticultural Research, Bengaluru 560089, Karnataka, India

[*]*Email: Dhananjaya.MV@icar.gov.in*

Introduction

Bottle gourd [*Lagenaria siceraria* (Mol) Standle; 2n = 22] commonly known as 'white flowered gourd' or 'calabash gourd' belongs to the family Cucurbitaceae. Its fruits are known for good nutritive value. It is rich source of vitamins, minerals and also possess diuretic, antioxidant, cardio protective properties (Barot *et al.* 2015). Acceptability for fruit shape varies with the region and segment. The Southern parts of the country prefer cylindrical shape, whereas North and north eastern regions prefer round to oblong shaped fruits. For achieving quick cultivar development in the desired shape, presence and utilization of resistance source in the desired fruit shape is well appreciated by the breeders in the development of varieties/hybrids with resistance. Keeping in view, ICAR-IIHR, Bengaluru has come out with genetic stocks having resistance to major biotic stress *i.e* gummy stem blight which is confronting the farmers. A promising genetic stock BG-114-1 with short, cylindrical shaped fruits was developed through individual plant selection from a open pollinated population collected and maintained in the germplasm. It was found to be resistant for gummy stem blight disease.

Morpho-agronomic characteristics

Plants are monoceious, annual vine with soft pubescence and produces white solitary flowers during evening hours. It takes an average of 41.38 days for first female flower appearance at 10.53 node. Fruits are green, short cylindrical with a length of 18.95 cm and circumference 25.66 cm. It also produces 5.75 number of fruits per plant with an marketable yield of 43.12t/ha.

Associated Characters and cultivation practices

Fruits are green, short cylindrical. It is also rich in Vit Ct and minerals like Iron, zinc, manganese and potash. Plants shows resistant reaction for gummy stem blight disease incidence across season with an average PDI of 9.01 under field screening and 9.25 under artificial screening. Bottle gourd is a warm season vegetable crop, it cannot with stand frost and usually prefers hot and moist climate for cultivation. Seeds are sown from January to February for summer crop, June to July for rainy season crop and October - November for Rabi season. Land should be prepared well before planting by repeated ploughing and harrowing, 20-25 tonnes of farm yard manure/ha should be broadcasted after the first ploughing.

Apply the recommended fertilizer mixture of 75kg N, 100 kg P_2O_5 and 35-70 K_2O/ha in two to three splits. Seeds are sown on raised beds of 15-30 cm height, 1-1.5m width during rainy season by maintaining a spacing of 1.6 m between the rows and 1m between the plants. Two to three seeds were sown per hill and covered with soil and light irrigation was given. Thinning of seedling should be done 25-30 days after sowing, retaining one healthy seedling per hill. Plants should be properly trained on the supporting structure to facilitate intercultural operations, thereby it reduces the incidence of pest and disease.

Traits of economic importance in (IC635410; INGR21145)

Sl. No	*Characters*	*BG-114-1*
Plant morphological and yield traits		
1.	Days to first female flower appearance	41.38
2.	Node on which first female flower appeared	10.53
3.	Number of female flower	10.33
4.	Number of fruits per plant	5.75
5.	Yield/ha	43.12
Fruit quality traits		
6.	Fruit length(cm)	18.95
7.	Fruit width(cm)	23.98
8.	Fruit weight(kg)	1.25
9.	Fruit shape	Short cylindrical
Nutrient and nutraceutical traits		
10.	Vit C (mg/100 g FW)	12.30
11.	Potassium (%)	5.54
12.	Iron (ppm)	74.00
13.	Manganese (ppm)	9.60
14.	Zinc (ppm)	23.00
Disease reaction		
15.	Gummy stem blight (PDI- Artificial screening)	9.25
16.	Gummy stem blight (PDI- Field screening)	9.01

References

Dhananjaya MV (2018). Production technology of vegetable crops (Bottle gourd)- A hand book, Edited by Sadashiva AT, Hebbar SS, Nair AK, and senthil Kumar M, IIHR, Bengaluru (INDIA), pp 26-28.

Amit B, Suneeta P, Smitha B and Prajapati JP (2015). Composition, Functional Properties and Application of Bottle Gourd in Food Products. *J Dairy Sci. Technol.,* **4(1):** 15–27.

35. BG-114-3 (IC635411; INGR21146), a bottle gourd [*Lagenaria siceraria* (Mol.) Standl.)] germplasm with resistance to gummy stem blight and medium cylindrical fruit

Dhananjaya MV*, Sandeep Kumar GM, Varalakshmi B and Mahesh B
ICAR-Indian Institute of Horticultural Research, Bengaluru 560089, Karnataka, India
**Email: Dhananjaya.MV@icar.gov.in*

Introduction

Bottle gourd [*Lagenaria siceraria* (Mol) Standle; 2n = 22] commonly known as 'white flowered gourd' or 'calabash gourd' belongs to the family Cucurbitaceae. Its fruits are known for good nutritive value. It is rich source of vitamins, minerals and also possess diuretic, antioxidant, cardio protective properties (Barot *et al.* 2015). Acceptability for fruit shape varies with the region and segment. The Southern parts of the country prefer cylindrical shape, where as North and north eastern regions prefer round to oblong shaped fruits. For achieving quick cultivar development in the desired shape, presence and utilization of resistance source in the desired fruit shape is well appreciated by the breeders in the development of varieties/hybrids with resistance. Keeping in view, ICAR-IIHR, Bengaluru has come out with genetic stocks having resistance to major biotic stress *i.e* gummy stem blight which is confronting the farmers. A promising genetic stock BG-114-3 with medium cylindrical shaped fruits was developed through individual plant selection from a open pollinated population collected and maintained in the germplasm. It was found to be resistant for GSB across the seasons with an average PDI of 6.22 under field screening and 9.45 under artificial screening.

Morpho-agronomic characterstics

Plants are monoecious, annual vine with soft pubescence and produces white solitary flowers during evening hours. It takes an average of 45.92 days for first female flower appearance at 9.24th node. Fruits are green, medium cylindrical with a length of 25.93 cm and circumference 25.66 cm. It also produces 7.16 number of fruits per plant with a marketable yield of 46.80t/ha.

Associated Characters and cultivation practices

It is also rich in Vit C and minerals like Iron, zinc, manganese and potash. It is a typical warm season vegetable, it cannot with stand frost and usually prefers hot and moist climate for cultivation. Seeds are sown from January to February for summer crop, June to July for rainy season crop and October - November for Rabi season. Land should be prepared well before planting by repeated ploughing and harrowing, 20-25 tonnes of farm yard manure/ha should be broadcasted after the first ploughing. Apply the recommended fertilizer mixture of 75 kg N, 100 kg P_2O_5 and 35-70 K_2O/ha in two to three splits. Seeds are sown on raised beds of 15- 30cm height, 1-1.5m width during rainy season by maintaining a spacing of 1.6 m between the rows and 1 m between the plants. Two to three seeds were sown per hill and covered with soil and light irrigation was given. Thinning of seedling should be done 25-30 days after sowing, retaining one healthy seedling per hill. Plants should be properly trained on the supporting structure to facilitate intercultural operations, thereby it reduces the incidence of pest and disease.

Traits of economic importance in (IC635411; INGR21146)

Sr. No	*Character*	*BG-114-3*
Morphological Traits		
1.	Days to first female flower appearance	45.92
2.	Node on which first female flower appeared	9.24
3.	Number of female flower	12.22
4.	Number of fruits per plant	7.16
5.	Yield /ha	46.80
Fruit quality traits		
6.	Fruit length (cm)	25.93
7.	Fruit circumference (cm)	25.66
8.	Fruit weight (kg)	1.09
9.	Fruit Shape	Medium Cylindrical
Nutrient and neutraceurtical traits		
10.	Vit C (mg/100 g FW)	8.20
11.	DPPH (mg/100g (FW) AEAC	10.55
12.	FRAP (mg/100g (FW) AEAC	13.77
13.	Potassium (%)	5.52
14.	Iron (ppm)	82.66
15.	Manganese (ppm)	12.30
16.	Zinc (ppm)	25.00
Disease reaction		
17.	Gummy stem blight disease (PDI-Artificial screening)	9.45
18.	Gummy stem blight disease (PDI-Field screening)	6.22

References

Dhananjaya MV (2018). Production technology of vegetable crops(Bottle gourd)- A hand book, Edited by Sadashiva AT, Hebbar SS, Nair AK, and senthil Kumar M, IIHR, Bengaluru (INDIA), pp 26-28.

Amit B, Suneeta P, Smitha B and Prajapati JP (2015). Composition, Functional Properties and Application of Bottle Gourd in Food Products, *Dairy Sci. Technol.* **4(1):** 15–27.

36. IC635413; BG-6-3 (IC635413; INGR21147), a bottle gourd [*Lagenaria siceraria* (Mol.) Standl.)] germplasm with resistance to powdery mildew and elongated straight fruit

Dhananjaya MV*, Sandeep Kumar GM, Varalakshmi B and Mahesh B

ICAR-Indian Institute of Horticultural Research, Bengaluru-560089, Karnataka, India

**Email: Dhananjaya.MV@icar.gov.in*

Introduction

Bottle gourd [*Lagenaria siceraria* (Mol) Standle; 2n = 22] commonly known as 'white flowered gourd' or 'calabash gourd' belongs to the family Cucurbitaceae. Its fruits are known for good nutritive value. It is rich source of vitamins, minerals and also possess diuretic, antioxidant, cardio protective properties (Barot *et al*. 2015). Acceptability for fruit shape varies with the region and segment. The Southern parts of the country prefer cylindrical shape, where as North and north eastern regions prefer round to oblong shaped fruits. For achieving quick cultivar development in the desired shape, presence and utilization of resistance source in the desired fruit shape is well appreciated by the breeders in the development of varieties/hybrids with resistance. Keeping in view, ICAR-IIHR, Bengaluru has come out with genetic stocks having resistance to major biotic stress *i.e* powdery mildew which is confronting the farmers.

Traits of economic importance in (IC635413; INGR21147)

Sl. No	*Character*	*BG-6-3*
Plant morphological and yield traits		
1.	Days to first female flower appearance	43.15
2.	Node on which first female flower appeared	8.21
3.	Number of female flower	15.55
4.	Number of fruits per plant	5.05
5.	Yield /ha	31.81
Fruit quality traits		
6.	Fruit length(cm)	34.75
7.	Fruit circumference (cm)	27.00
8.	Fruit weight(kg)	1.05
9.	Fruit shape	Elongated straight
Disease reaction		
10.	Powdery mildew	11.11(PDI) 266.67 (AUDPC)

A promising genetic stock BG-6-3 with medium elongated straight shape was developed through individual plant selection from a open pollinated population collected and maintained in the germplasm. It was found to be resistant for powdery mildew.

Morpho-agronomic characterstics

Plants are monoceious, annual vine with soft pubescence and produces white solitary flowers during evening hours. It takes an average of 43.15 days for first female flower appearance at 8.21 node. Fruits are green, medium elongated straight with a length of 34.75 cm and circumferance of 27.00 cm. It also produces 5.05 number of fruits per plant with an marketable yield of 31.81t/ha.

Associated Characters and cultivation practices

Fruits are medium, elongated straight with green in colour. Plants shows resistant reaction for powdery mildew disease under artificial screening. It is a typical warm season vegetable, it cannot with stand frost and usually prefers hot and moist climate for cultivation. Seeds are sown from January to February for summer crop, June to July for rainy season crop and October - November for Rabi season. Land should be prepared well before planting by repeated ploughing and harrowing, 20-25 tonnes of farm yard manure/ha should be broadcasted after the first ploughing.

Apply the recommended fertilizer mixture of 75 kg N, 100 kg P_2O_5 and 35-70 K_2O/ha in two to three splits. Seeds are sown on raised beds of 15- 30cm height, 1-1.5m width during rainy season by maintaining a spacing of 1.6 m between the rows and 1m between the plants. Two to three seeds were sown per hill and covered with soil and light irrigation was given. Thinning of seedling should be done 25-30 days after sowing, retaining one healthy seedling per hill. Plants should be properly trained on the supporting structure to facilitate intercultural operations; thereby it reduces the incidence of pest and disease.

References

Dhananjaya MV (2018). Production technology of vegetable crops(Bottle gourd)- A hand book, Edited by Sadashiva AT,

Hebbar SS, Nair AK, and senthil Kumar M, IIHR, Bengaluru (INDIA), pp 26-28.

Amit B, Suneeta P, Smitha B and Prajapati JP (2015) Composition, Functional Properties and Application of Bottle Gourd in Food Products. *Journal of Dairy Science and Technology*, **4(1):** 15-27.

37. IC635412; BG-95 (IC635412; INGR21148), a bottle gourd (*Lagenaria siceraria* (Molina) Standl.) germplasm with resistance to gummy stem blight and round shaped fruit

Dhananjaya MV*, Sandeep Kumar GM, Varalakshmi B and Mahesh B

ICAR-Indian Institute of Horticultural Research, Bengaluru-560089, Karnataka, India

**Email: Dhananjaya.MV@icar.gov.in*

Introduction

Bottle gourd [*Lagenaria siceraria* (Mol) Standle; 2n = 22] commonly known as 'white flowered gourd' or 'calabash gourd' belongs to the family Cucurbitaceae. Its fruits are known for good nutritive value. It is rich source of vitamins, minerals and also possess diuretic, antioxidant, cardio protective properties (Barot *et al.* 2015). Acceptability for fruit shape varies with the region and segment. The Southern parts of the country prefer cylindrical shape, whereas north and north eastern regions prefer round to oblong shaped fruits. For achieving quick cultivar development in the desired shape, presence and utilization of resistance source in the desired fruit shape is well appreciated by the breeders in the development of varieties/hybrids with resistance. Keeping in view, ICAR-IIHR, Bengaluru has come out with genetic stocks having resistance to major biotic stress *i.e* gummy stem blight which is confronting the farmers. A promising genetic stock BG-95 with round shape was developed through individual plant selection from a open pollinated population collected and maintained in the germplasm. It was found to be resistant for gummy stem blight disease.

Traits of economic importance in (IC635412; INGR21148)

Sl. No	*Character*	*BG-95*
Morphological Traits		
1.	Days to first female flower appearance	53.55
2.	Node on which first female flower appeared	14.01
3.	Number of female flower	12.67
4.	Number of fruits per plant	4.79
5.	Yield /ha	36.78
Fruit quality traits		
6.	Fruit length(cm)	14.25
7.	Fruit circumference (cm)	41.48
8.	Fruit weight(kg)	1.28
9.	Fruit shape	Round
10.	Fruit firmness(kg/cm2)	10.69
11.	Pericarp thickness(mm)	15.18
11.	Gummy stem blight disease (PDI- Artificial screening)	8.89
12.	Gummy stem blight disease (PDI- Field screening)	9.27

Morpho-agronomic characteristics

Plants are monoecious, annual vine with soft pubescence and produces white solitary flowers during evening hours. It takes an average of 53.55 days for first female flower appearance at 14.01 node. Fruits are green, round with a length of 14.25 cm and circumference 41.48cm. It also produces 4.79 number of fruits per plant with an marketable yield of 36.78t/ha.

38. VRT12-1-3-2 (IC640702; INGR21149), a tomato (*Solanum lycopersicum* L.) germplasm with broad spectrum resistance to tomato leaf curl virus possessing Ty-3 gene along with uniform ripening fruits and greater combining ability

Prasanna HC[1*], Yerasu Suresh Reddy[2] and Major Singh[3]

[1]*ICAR-Indian Institute of Horticultural Research, Bangalore -560089, Karnataka, India*

[2]*ICAR-Indian Institute of Vegetable Research, Varanasi–221305, Uttar Pradesh, India*

[3]*ICAR-Directorate of Onion and Garlic Research, Pune-410505, Maharashtra, India*

**E-mail: yerasusureshreddy@gmail.com*

Tomato (*Solanum lycopersicum*) is one of the major vegetable crops consumed and cooked in different forms/ ways worldwide. Tomato is vulnerable to a wide range of different pathogens leading to significant yield loss every year world over. Among them, *Tomato leaf curl virus* (ToLCV), a *Begomovirus* from the *Geminiviridae* family, transmitted by

whiteflies (*Bemisia tabaci*) is a major challenge in tomato production and can cause 100% yield loss if young tomato plants are infected. Breeding and cultivating ToLCV resistant cultivars is a proven strategy to minimize the loss caused by the disease.

Since inception, ICAR-Indian Institute of Vegetable research, Varanasi is working on development of ToLCV resistant tomato cultivars. VRT12-1-3-2 is one such product from resistant breeding efforts and has good resistance against ToLCV. VRT12-1-3-2 was developed through marker assisted selection from F_2 populations of a cross between FLA478-6-1-11 × CLN2498C and FLA478-6-1-11 × CLN1621E. The lines CLN2498C and CLN1621E carry *Ty-2* gene. The line FLA478- 6-1-11 carry *Ty-3* gene (Prasanna *et al.*, 2015). *Ty-3* gene is a partially dominant gene and was introgressed into cultivated tomato from *S. chilense* (LA2779) (Verlaan *et al.* 2013).

The tomato genotype VRT12-1-3-2 has *Ty-3* gene. The tomato line VRT12-1- 3-2 showed a high level of resistance to ToLCV. The response of the line to infection by a monopartite (ToLCBV) and a bipartite (ToLCNDV) virus was tested using both field tests and agroinoculation based disease screens. The severity of the disease symptoms (DSI) was assessed at 30 and 60 days following agroinoculation. Tomato line VRT12-1-3-2 had an average DSI of 0.5 to 0.6 and 0.9 in response to ToLCBV and ToLCNDV infection, respectively at 60 DPI. Susceptible genotype Punjab chuuhara showed only moderate susceptibility at 30 days post inoculation (DPI) of Tomato Leaf Curl Bangalore Virus (DSI of 3) but showed higher disease severity at 60DPI (DSI of 3.6). The average DSI of Kashi Vishesh was ≥ 3.1 at different stages in both agroinoculation and field tests. The tomato line VRT2-2-3-1 showed high level of resistance in the field even at 90 days after transplanting (Prasanna *et al.*, 2015).

The *Ty-3* containing tomato line VRT12-1-3-2 recorded an average fruit yield of 4.07 kg. The fruits of the line VRT12-1-3-2 are circular in shape with pericarp thickness of 0.49 cm and with an average locule number of 2.47 per fruit. VRT12-1-3-2 has greater combining ability. With ToLCV resistance, uniform fruit ripening, desirable horticultural characters, and combining ability, VRT12-1-3-2 can be used in different tomato ToLCV resistance breeding programmes like the line can be used as a parent in hybrid development programmes and as source of *Ty-3* gene in gene pyramiding programmes with other economically useful genes.

References

Prasanna H, Kashyap SP, Krishna R, Sinha DP, Reddy S and Malathi VG (2015). Marker assisted selection of Ty-2 and Ty-3 carrying tomato lines and their implications in breeding tomato leaf curl disease resistant hybrids. *Euphytica,* **204:** 407-418.

Verlaan MG, Hutton SF, Ibrahem RM, Kormelink R, Visser RG and Scott JW (2013). The tomato yellow leaf curl virus resistance genes Ty-1 and Ty-3 are allelic and code for DFDGD-class RNA-dependent RNA polymerases. *PLoS Genet*. **9(3):** e1003399. *DOI: 10.1371/journal.pgen.1003399*

39. VRT2-2-3-1 (HCP/YSR-2/) (IC637249; INGR21150), a tomato (*Solanum lycopersicum* L.) germplasm with broad spectrum resistance to tomato leaf curl virus and possessing Ty-3 gene along with green fruit shoulder and greater combining ability

Prasanna HC[1*], Yerasu Suresh Reddy[2] and Major Singh[3]

[1]*ICAR-Indian Institute of Horticultural Research, Bangalore-560089, Karnataka, India*

[2]*ICAR-Indian Institute of Vegetable Research, Varanasi-221305, Uttar Pradesh, India*

[3]*ICAR-Directorate of Onion and Garlic Research, Pune -410505, Maharashtra, India*

[*]*E-mail: yerasusureshreddy@gmail.com*

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops in the world along with potato and onion. Tomato (yellow) leaf curl disease (TYLCD/ ToLCD) is a major biotic constraint in tomato production and can cause 100% yield loss if young tomato plants are infected. ToLCD caused by begomoviruses and transmitted by whitefly (*Bemisia tabaci*). Among different available strategies, cultivating ToLCD resistant cultivars is the most effective, economical and environmentally safe strategy to manage the disease.

With an aim to develop ToLCV resistant cultivars, resistant breeding programme was initiated at ICAR-Indian Institute of Vegetable research, Varanasi. One of the products of the programme was tomato elite line VRT2-2-3-1. It has good resistance against ToLCV. VRT2-2-3-1 was developed through marker assisted selection from F_2 populations of a cross between FLA478-6-1-11 × CLN2498C and FLA478-6-1-11 × CLN1621E. The lines CLN2498C and CLN1621E carry *Ty-2* gene. The line FLA478-6-1-11 carry *Ty-3* gene (Prasanna *et al.*, 2015). VRT2-2-3-1 carries *Ty-3* gene which is a partially dominant gene.

The response of this line to infection by a monopartite (ToLCBV) and a bipartite (ToLCNDV) virus was tested using both field tests and agro inoculation based disease screens. The tomato line VRT2-2-3-1 showed a high level of resistance to both ToLCBV and ToLCNDV. The severity of the disease symptoms (DSI) was assessed at 30 and 60 days following agro inoculation. VRT2-2-3-1 showed similar disease

response in two independent inoculation experiments. Susceptible genotype Punjab chuuhara showed only moderate susceptibility at 30 days post inoculation (DPI) of *Tomato Leaf Curl Bangalore Virus* (DSI of ≤ 3) but showed higher disease severity at 60 DPI (DSI > 3.6). The average DSI of Kashi Vishesh was ≥ 3.1 at different stages in both agro inoculation and field tests. The tomato line VRT2-2-3-1 showed high level of resistance in the field even at 90 days after transplanting (Prasanna *et al*., 2015).

The tomato line VRT2-2-3-1 recorded an average fruit yield of 4.94 kg. The fruits of the line VRT2-2-3-1 showed high pericarp thickness (0.67 cm) with an average locule number of 2.7 per fruit. Fruit of VRT2-2-3-1 are round and has green shoulder. VRT2-2-3-1 has good combining ability. With ToLCV resistance, desirable horticultural characters, green shoulder and combining ability, VRT2-2-3-1 can be used in different tomato ToLCV resistance breeding programmes like the line can be used as a parent in hybrid development programmes especially for green shoulder market segment and as source of *Ty-3* gene in gene pyramiding programmes with other economically useful genes.

References

Prasanna H, Kashyap SP, Krishna R, Sinha DP, Reddy S and Malathi VG (2015). Marker assisted selection of Ty-2 and Ty-3 carrying tomato lines and theirimplications in breeding tomato leaf curl disease resistant hybrids. *Euphytica*, **204:** 407-418.

Verlaan MG, Hutton SF, Ibrahem RM, Kormelink R, Visser RG, Scott JW, Edwards JD, and Bai Y (2013). The Tomato yellow leaf curl virus resistance genes Ty-1 and Ty-3 are allelic and code for DFDGD-class RNA-dependent RNA polymerases. *PLoS Genet*, **9 (3):** e1003399. DOI: 10.1371/journal.pgen.1003399

40. VRT6-1-4 (HCP/YSR-5/) (IC637252; INGR21151), a tomato (*Solanum lycopersicum* L.) germplasm with broad spectrum resistance to tomato leaf curl virus possessing Ty-2 and Ty-3 genes pyramided along with uniform ripening of fruits.

Prasanna HC[1*], Yerasu Suresh Reddy[2] and Major Singh[3]

[1]*ICAR-Indian Institute of Horticultural Research, Bangalore -560089, Karnataka, India*

[2]*ICAR-Indian Institute of Vegetable Research, Varanasi–221 305, Uttar Pradesh, India*

[3]*ICAR-Directorate of Onion and Garlic Research, Pune - 410 505, Maharashtra, India*

**E-mail: yerasusureshreddy@gmail.com*

The whitefly-transmitted tomato yellow leaf curl virus (ToLCV) is one of the major hurdle in successful cultivation of tomato. Breeding tomatoes with resistance against whiteflies and/or viruses is the most effective method to minimize the damage caused by ToLCV and to avoid excessive use of insecticides. Wild tomato relatives are the main sources for ToLCV resistance. To date, six resistance genes (Ty-1, Ty-2, Ty-3, Ty-4, ty-5, and Ty-6) identified from several wild tomato species have been well studied (Ji *et al*., 2007; Hutton and Scott, 2014). Among them, Ty-1, Ty-2, and Ty-3 are the main sources of resistance for tomato breeding programs. Ty-1 and Ty-3 are alleles of the same gene.

Tomato line VRT6-1-4 with *Ty2* and *Ty3* genes pyramided was developed at ICAR-Indian Institute of Vegetable research, Varanasi. This tomato genotype has high ToLCV resistance and was developed through marker assisted selection from F2 populations of cross between FLA478-6-1-11 × CLN2498C and FLA478-6-1-11 × CLN1621E. The lines CLN2498C and CLN1621E carry *Ty-2* gene. The line FLA478-6-1-11 carries *Ty-3* gene (Prasanna *et al*., 2015).

The tomato line VRT6-1-4 showed a high level of resistance in both field tests and agroinoculation based disease screens. Tomato-infecting begomovirus species used in agroinoculation comprised bipartite Tomato leaf curl New Delhi virus (ToLCNDV) and Tomato leaf curl Palampur virus (ToLCPalV), and a monopartite Tomato leaf curl Bangalore virus (ToLCBV). The severity of the disease symptoms (DSI) was assessed at 30 and 60 days following agroinoculation and at 45 and 90 days after transplanting in field conditions. Tomato line VRT6-1-4 had an average DSI of and 0.2 in response to ToLCNDV and ToLCPalV infection, respectively at 60 DPI. There were no ToLCV symptoms even after 60 days following agroinoculation with monopartite ToLCBV. Cultivars Kashi Vishesh and Punjab Chhuhara were used as susceptible checks and they showed disease symptoms in all the experiments. High disease severity index (DSI) of ≥ 3.7 was recorded on both of these cultivars at 60 DPI. The line carrying Ty-2 showed moderate resistance to ToLCBV (DSI <2) with moderate leaf curling symptoms, but it was susceptible to both bipartite ToLCNDV (DSI 2.9) and ToLCPalV (DSI 2.2) at 60 DPI. The tomato line VRT6-1-4 showed high level of resistance under natural epiphytotic conditions in field even at 90 days after transplanting (Prasanna *et al*., 2015).

The pyramided line VRT6-1-4 recorded an average fruit yield of 3.22 kg/plant. The fruits of pyramided line D6-1-4 showed high pericarp thickness of 0.5-cm with an average locule number of 3.8 per fruit and total soluble solids (TSS) of 4.8 Brix. Fruit diameter and fruit length recorded on the fruits of VRT6-1-4 indicated that the high-yielding pyramided line VRT6-1-4 had round to high round fruits that ripen uniformly. VRT6-1-4 can be used in the tomato ToLCV resistance breeding programmes. It can be used as inbred

in hybrid development programmes especially for uniform ripening market segment and as a source of *Ty-2 +Ty-3* in gene pyramiding programmes to combine other Ty genes.

References

Hutton SF and Scott JW (2014). Ty-6, a major begomovirus resistance gene located on chromosome 10. *Report of the tomato genetics cooperative*. **64:** 14–18.

Ji Y, Scott JW, Hanson P, Graham E and Maxwell DP (2007b). Sources of resistance, inheritance, and location of genetic loci conferring resistance to members of the tomato-infecting begomoviruses. In: Czosnek H, ed. Tomato Yellow Leaf Curl Virus Disease: Management, Molecular Biology, Breeding for Resistance. Dordrecht, The Netherlands: *Springer*, 343–62.

Prasanna HC, Sinha DP, Rai GK, Krishna R, Kashyap S P, Singh N K and Singh M. and Malathi VG. (2015) Pyramiding Ty-2 and Ty-3 genes for resistance to monopartite and bipartite tomato leaf curl viruses of India. *Plant Pathology*. **64 (2):** 256- 264.

41. Selection-24 (IC640703; INGR21152), a natural dwarf variant of tomato (*Solanum lycopersicum* L.)

K Chandrashekar, Swati Saha*, Raj Verma, Savarni Tripathi, Om Prakash Meena and GK Mahapatro

ICAR-IARI Regional Station, Pune-411067, Maharashtra, India

Email: swatisaha1980@gmail.com

Introduction

Selection-24 (Seln-24) a natural dwarf variant identified and stabilized from a segregating population of tomato variety Pusa Gaurav. The line is dwarf in nature with mean plant height of about 40-45 cm. Line has shown consistent field tolerance to whitefly and leaf curl virus disease (Chandrashekar *et al.,* 2019 and Saha *et al*., 2021). The attributes of Seln-24 *viz*., determinate dwarf bushy plants with moderate green foliagecover, attractivebullet shaped immature fruits and attractive deep red color elliptical (egg-shaped) ripe fruits, makes it highly suitable for terrace and vertical gardening. Due to its compact and dwarf nature, Seln-24 requires minimum staking and training.

Morpho-agronomic characteristics

Seln-24 showed significant reduction in plant height (42 cm) which was up to 4 times less compared to parental line Pusa Gaurav ranging between 130.9 to 140.72 cm (Table 1). Plants are determinate, dwarf bushy with moderate green foliage cover. Leaf shape is slightly serrated, green. Number of flowers per truss are more than six, number of fruits per

Table 1: Comparison of L-24 for plant height, yield, pest and disease incidence along with the checks (2016-2018)

S. No.	*Line*	*Plant height (CM)*	*Whitefly (No./leaf)*	*Thrips (No./sample)*	*Leaf curl (% incidence)*	*Yield (KG/Plant)*	*TSS*
				2016			
1	Seln-24	53.01 ± 2.51	0.08 ± 0.0	0.58 ± 0.04	7.4 ± 1.2	1.58 ± 0.11	3.72 ± 0.19
2	Pusa Rohini	150.37 ± 5.72	0.25 ± 0.1	0.42 ± 0.04	23.3 ± 5.8	1.90 ± 0.90	4.06 ± 0.06
3	L-29	209.30 ± 4.93	0.17 ± 0.01	0.64 ± 0.22	9.1 ± 4.0	2.34 ± 0.67	4.26 ± 0.19
4	L-33	139.70 ± 2.13	0.5 ± 0.19	0.59 ± 0.0	0.0	1.76 ± 0.22	3.8 ± 0.17
5	Arka Rakshak	169.31 ± 5.30	0.2 ± 0.2	0.42 ± 0.04	7.25 ± 2.5	4.21 ± 1.33	4.96 ± 0.04
6	Pusa Gaurav	130.05 ± 13.0	0.25 ± 0.0	0.82 ± 0.04	10.5 ± 3.8	2.75 ± 0.14	3.8 ± 0.10
				2017			
1	Seln-24	38.0 ± 2.97	1.00 ± 0.01	2.80 ± 0.33	2.10 ± 1.96	1.36 ± 0.27	3.60 ± 0.3
2	Pusa Rohini	173.74 ± 12.01	1.67 ± 0.2	3.33 ± 0.33	41.43 ± 7.78	2.06 ± 0.23	4.0 ± 0.0
3	L-29	251.97 ± 21.67	0.20 ± 0.05	3.67 ± 0.67	3.7 ± 2.56	1.14 ± 0.09	4.17 ± 0.23
4	L-33	170.69 ± 3.05	1.67 ± 0.25	3.00 ± 0.67	0.0	2.19 ± 0.20	3.73 ± 0.29
5	Arka Rakshak	217.42 ± 4.93	3.33 ± 0.1	4.67 ± 0.42	12.63 ± 2.78	2.78 ± 0.76	5.0 ± 0.0
6	Pusa Gaurav	140.72 ± 5.74	1.67 ± 0.1	3.0 ± 0.33	22.5 ± 6.4	1.82 ± 0.13	3.8 ± 0.20
				2018			
1	Seln-24	32.99 ± 2.31	1.67 ± 0.67	2.13 ± 0.66	6.86 ± 1.7	1.54 ± 0.20	3.93 ± 0.07
2	Pusa Rohini	171.20 ± 10.19	1.33 ± 0.33	3 ± 0.93	11.46 ± 1.0	1.84 ± 0.14	4.10 ± 0.10
3	L-29	262.64 ± 23.93	0.3 ± 0.1	3.8 ± 1.04	3.79 ± 0.8	1.68 ± 0.18	4.47 ± 0.24
4	L-33	181.86 ± 11.86	3.3 ± 0.6	4.53 ± 1.15	0.0	2.31 ± 0.33	4.0 ± 0.12
5	Arka Rakshak	218.95 ± 5.44	1.67 ± 0.33	3 ± 1.24	6.84 ± 1.96	3.52 ± 0.18	4.93 ± 0.19
6	Pusa Gaurav	133.10 ± 7.54	1.33 ± 067	2.60 ± 0.66	8.2 ± 2.5	2.63 ± 0.28	3.4 ± 0.14

cluster is more than five, average number of fruits per plant vary from 50-55. Fruits are smooth, elliptical (egg-shaped) and borne in clusters, uniformly red and thick skinned with two locules. Average yield per plant is around 1.5-2.0 kg/ plant. TSS (3.6 Brix) and better keeping quality at room temperature.

Mean fruit weight varies from 50-60 g. Fruit length more than 5 cm while fruit diameter is 4 cm. The vitamin C content is quite higher i.e. 43 mg/100gFW (Table 2). Seln-24 evaluated for performance under terrace gardening in different sized pots. When grown in large pots each plant yielded on an average 2.0 kg fruits/plant.

Seln-24 has consistently showed field tolerance to whiteflies and leaf curl disease during the years 2016, 2017, 2018-19 (Table 1) and 2019-20 (which was comparable to standard leaf curl resistant check "Arka Rakshak", a parent line "Pusa Gaurav" and diseases resistant check lines (L-29 and L-33). The disease tolerance observed during the year 2016, 2017 and 2018 in Seln-24 (7.4, 2.10 and 3.79% disease incidence) which was similar to Arka Rakshak (7.25, 12.63 and 6.84%) and much higher than popular variety Pusa Rohini (23.3, 41.43 and 11.46%) (Table1). The population of whiteflies recorded on Seln-24 was 0.58, 2.80 and 2.13 whiteflies/leaf respectively, in the year 2016, 2017 and 2018. The population of whiteflies recorded on standard check Pusa Rohini was 0.42, 3.33 and 3.0 and on Arka Rakshak it was 0.4 4.67 and 3.0 whiteflies/leaf respectively. Similar tolerance to vector and leaf curl virus was also observed during 2019-20 also.

Associated characters and cultivation practices

Average plant height observed for Seln-24 is the most suitable for pot culture as well as high density planting. Huge cost is involved in staking sticks/stumps, steel wires, gunny twine/rope and labour for twining tomato plants. The cost involve in this activity varies from 7 to 11%. Use of dwarf determinate variety Seln-24 in the near future will lead to savings in cost of cultivation for the staking in tomato.

Since the line is dwarf type staking operation is not required which saves a lot of labour and expenditure.

Table 2: Mean performance of yield, and quality traits of various tomato genotypes

S. No.	*Accessions*	*Plant height (cm)*	*No of branches/ plant*	*No of flowers /truss*	*No of fruits/ cluster*	*No of fruits/ plant*	*Yield /plant (kg)*	*Yield (t/ha)*	*IFW (g)*	*FL (cm)*	*FD v(cm)*	*PT (cm)*	*FC (cm)*	*TSS° Brix*	*Vitamin C (mg/100g FW)*
1	Arka Rakshak	201.84	5.93	6.40	5.93	81.8	3.31	81.90	81.53	5.31	4.88	0.60	3.91	3.40	19.38
2	Pusa Rohini	165.10	6.73	6.46	6.27	85.1	1.86	45.94	71.25	4.45	5.15	0.65	4.16	3.45	28.63
3	Pusa Gaurav	134.62	7.33	4.26	5.67	64.6	2.29	56.76	48.11	4.9	4.51	0.55	3.50	3.60	40.47
4	Seln-29	241.30	8.13	7.40	5.53	53.27	1.81	44.78	34.78	4.16	3.98	0.51	3.00	2.73	21.62
5	Seln-33	164.08	7.93	6.13	6.20	47.7	2.05	50.69	35.41	4.48	3.93	0.55	2.86	3.13	14.78
6	Seln-24	41.53	7.13	6.40	5.40	55.5	1.18	29.33	53.58	5.48	4.13	0.56	3.06	2.93	43.88
	Mean	158.07	7.19	6.17	5.83	64.66	2.08	51.56	54.11	4.79	4.43	0.57	3.41	3.20	28.12
	CD	23.58	NS	NS	NS	12.56	0.75	18.70	6.18	0.43	0.4	NS	0.29	NS	4.98
	CV	8.20	15.85	16.75	18.53	21.58	40.25	40.26	6.28	5.02	5.06	13.69	4.71	12.03	9.74

(FL-Fruit length, FD- Fruit Diameter, PT- Pericarp Thickness, FC- Fruit Cavity, IFW- Individual Fruit Weight, TSS- Total Soluble Solids)

References

K Chandrashekar, Swati Saha**,** Savarni Tripathi, Raj Verma and SK Sharma (2019). A Dwarf Tomato Line Suitable for Ornamental/ Kitchen Gardening. *First Vegetable Science Congress on Emerging Challenges in Vegetable Research & Education (VEGCON-2019) 1-3 February, 2019, Jodhpur Rajasthan*, Pg-174.

Swati Saha, K. Chandrashekar, Raj Verma and Savarni Tripathi (2021). Performance evaluation of natural dwarf mutant tomato suitable for vertical gardening. *Indian Journal of Horticulture*, **78(2):** 169- 174.

42. CARI Brinjal 2 (IC640704; INGR21153), a brinjal (*Solanum melongena* L.) germplasm with resistance to bacterial wilt disease caused by *Ralstonia solanacearum*

Naresh Kumar[1], Pankaj Kumar Singh[1*], Raj Kumar Gautam[2], Krishna Kumar[3], Ajanta Birah[4], K Sakthivel[5], SK Zamir Ahmad[1], K Venkatesan[1] and B Augustine Jerard[1]

[1]*ICAR-Central Island Agricultural Research Institute, Port Blair-744105, Andaman and Nicobar Islands, India*

[2]*ICAR-National Bureau of Plant Genetic Resources, Pusa Campus-110012, New Delhi, India*

[3]*Pt. Deen Dayal Upadhya Collge of Horticulture and Forestry, Dr. RPAU, Samastipur-848125, Bihar, India*

[4]*ICAR-Indian Agricultural Research Institute, Pusa Campus-110012, New Delhi, India*

[5]*ICAR-Indian Institute of Oilseeds Research, Hyderabad, 500030, Telangana, India*

[*]*Email: pksingh1975@gmail.com*

Brinjal or eggplant (*Solanum melongena* L.) is severely affected by bacterial wilt caused by Ralstonia solanecearum in India. Resistant varieties are most effective and suitable option to reduce crop losses from bacterial wilt but knowledge of resistance mechanism and development of resistant varieties is difficult and time taking job. Further, most of the brinjal germplasm in India showed susceptible reaction under rainfed low land conditions of Andaman and Nicobar Islands. Thus, the present study was carried out at ICAR-CIARI, Port Blair during 2010-2018 to understand the genetic behaviour of bacterial wilt resistance in brinjal and developed a bacterial wilt resistant variety by pedigree selection method where crosses made between "Pusa Purple Long" (5) x "CARI Brinjal 1" (R) in Island conditions. Single F, plant progenies were advanced up to F4 and recorded the reaction of segregating population in the sick plots_ In this process, one bacterial wilt resistant brinjal line CARI Brinjal 2 has been identified as medium tall, semi-spreading plant type with profuse branching habit and smooth stems. Leaves are smooth medium leaves which are green in colour. Fruits are oblong in shape, purple in colour, medium compact, pendent position and medium seeded. Seeds of this line are medium in shape and size and light yellow in colour. It gives fruit yield up to 16 t/ha under rainfed conditions of Andaman and Nicobar Islands during Rabi season.

In most cultivated peppers (*Capsicum* spp.) the fruit adheres tightly to the calyx when ripe and the pedicel remains attached to the fruit when harvested. An economical problem with most cultivated pepper fruit used for processing is the tight adherence of the fruit to the receptacle which results in attached woody pedicels and green calyxes on the fruits and it impart off-colour, lower the quality and high cost during processing. Dry chilli industries are also suffering with labor shortage for the removal of stalk or pedicel before processing. Consequently, processors bid 5 to 10% less to the farmers chilli produce which is pedicel attached chilli by considering the quality. This identified chilli germplasm/ line, NPC-3 (IC631915) is erect bearing and will overcome the problem of removal of stems/stalks from the fruits and increases quality of the product. At the time of harvesting, this line expresses the easy separable character of stems/ stalks from the fruits, the fruits will be separated easily from the plants without stalk. Identified germplasm is stalk less line which facilitate to easy separation of fruits from stalk. So that, cost of post harvest handling and processing can be reduced.

NPC-3 (1.44 t/ha) was shown stability for the trait and yielded highest across the years in the same location and over the check (1.29 t/ha). The genotype NPC-3 was shown moderately resistannce for the chilli leaf curl, good capsaicin content of 19217 SHU and having good red color count of 138.5 ASTA.

43. NPC-3 (IC631915; INGR21154), a chilli (*Capsicum annuum* L.) germplasm with non-persistent calyx, erect bearing habit and high yield

Prabhudeva Ajjappalavara[1*], HB Patil[2], TB Allolli[2], N Basavaraja[2], DR Patil[3], KM Indiresh[2], MH Tatagar[4] and L Hegde[5]

[1]*University of Horticultural Sciences, Horticultural Research & Extension Center, Devihosur-581110, Karnataka, India*

[2]*College of Horticulture, Bagalkot-587104, Karnataka, India*

[3]*Main Horticulture Research & Extension Center, Bagalkot-587104, Karnataka, India*

[4] *College of Horticulture, Sirsi-581402, Karnataka, India*

[5] *College of Horticultural Engineering & Food Technology, Devihosur-581110, Karnataka, India*

[*]*Email: prabhudev.ajjappalavar@uhsbagalkot.edu.in*

44. NPC-5 (IC631916; INGR21155), a chilli (*Capsicum annuum* L.) germplasm with non-persistent calyx, pendent bearing habit, high yield and resistance to chilli leaf curl complex

Prabhudeva Ajjappalavara[1*], HB Patil[2], TB Allolli[2], KM Indiresh[2], N Basavaraja[2], Revanappa[3], Abdul Kareem[4] and Pallavi HM[2]

[1]*University of Horticultural Sciences, Horticultural Research & Extension Center, Devihosur-581110, Karnataka, India*

[2] *College of Horticulture, Bagalkot-587104, Karnataka, India*

[3] *Horticulture Extension Education Unit, Yadagir- 585201, Karnataka, India*

[4] *College of Horticulture, Sirsi-581402, Karnataka, India*

[5] *College of Horticulture, Mysore- 571130, Karnataka, India*

[*]*Email: prabhudev.ajjappalavar@uhsbagalkot.edu.in*

In most cultivated peppers (*Capsicum* spp.) the fruit adheres tightly to the calyx when ripe and the pedicel remains attached to the fruit when harvested. An economical problem with most cultivated chilli fruit used for processing is the tight adherence of the fruit to the receptacle which results in attached woody pedicels and green calyxes on the fruits and it impart off-colour, lower the quality and high cost during processing. Dry chilli industries are also suffering with labor shortage for the removal of stalk or pedicel before processing. This identified chilli germplasm/ line, NPC-5 (IC631916) is pendent bearing and overcome the problem of removal of stems/stalks from the fruits and increases quality of the product. This line is having the easy separable character of stems/stalks from the fruits. While harvesting, the fruits will be separated easily from the plants without stalk. Identified germplasm is stalk less line which facilitate to easy separation of fruits from stalk.

So that, cost of post harvest handling and processing can be reduced. NPC-5 (1.48 t/ha) was shown stability for the trait and yielded highest across the four years in the same location and over the Sapan F1 hybrid Dandicut (1.29 t/ha). The NPC-5 was recorded resistance for the chilli leaf curl and high yield.

Traits of economic importance in (IC631916; INGR21155)

Characters	*Expression*
Growth habit	Upright
Bearing habit	Solitary
Fruit length (cm)	Medium (6.5 to 7.5)
Fruit width (cm)	Medium (1.10 to 1.4)
Flower position/orientation	Pendent/drooping
Colour at ripen maturity	Red
Capsaicin content (SHU)	14494 (0.0.09 %)
Ripened fruit Color (ASTA)	77.5
Oleoresin recovery (%)	15.0 to 16.50
Pedicel with fruit	Non persistent
Pedicel with stem	Persistent
Yield per plant (g)	46 to 55
Yield potentiality (t/ha)	1.30 to 2.30
Reaction to chilli leaf curl disease	Moderately resistance
Suitability	Dry chilli industry

45. TCGS-1862 (IC640705; INGR21156), a groundnut (*Arachis hypogaea* L.) germplasm with stay green character and resistance to stem rot, late leaf spot and rust

K John, RP Vasanthi, A Srividhya, M Subbarao, K Viswanath, C Kirankumar Reddy and L Prasanthi

Institute of Frontier Technology, Regional Agricultural Research Station, Tirupati-517502, Andhra Pradesh, India

[*]*Email: johnlekhana@rediffmail.com*

Introduction

The low productivity in groundnut is attributed to several production constraints. Increased groundnut production can be achieved by using high yielding varieties and adopting improved crop management practices. Seed and seedling diseases cause severe seedling mortality resulting in "patchy" crop stand and ultimately reduced yields. Among seed and seedling diseases, collar rot and stem rot diseases are considered economically important diseases of groundnut in India and world as well. 80% of groundnut area is under rainfed situation in Andhra Pradesh. It is cultivated in light sandy soils where the incidence of soil borne diseases (Collar rot and stem rot) is more. It is difficult to control the soil borne diseases through foliar spray. The yield losses of over 25% have been reported by Mayee and Datar (1988) in Maharashtra. Collar rot disease causes considerable seedling mortality in early stages of crop growth. Crop yield is directly affected by reduction in the stand of the crop. These soil borne diseases reduce pod yield and haulms yield. Late

Table 1: Mean performance of the groundnut germplasm accession TCGS- 1862 (INGR 21156)

S.No	Character	2016	2016-17	2017	Mean
1.	Plant height (cm)	26.6	17.6	32.2	25.5
2.	Days to 50% flowering	32	30	29	30.3
3.	Days to maturity	120	117	99	112.0
4	Final plant stand ('000'/ha)	291	197	202	230.0
5	No. of primary branches/ plant	8.8	4.4	5.2	6.1
6	No. of secondary branches/ plant	3.2	2.8	4.0	3.3
7	100 pod weight (g)	54	82	77	71.0
8	100 kernel weight (g)	22	37	30	29.7
9	Sound mature kernel per cent	82	84	86	84.0
10	Shelling per cent	59	64	64	62.0
11	Haulm yield (kg/ha)	4550	2719	2746	3338
12	Pod yield (kg/ha)	1888	3181	2559	2543
13	Kernel yield (kg/ha)	1114	2036	1639	1596

Table 2: Reaction of groundnut germplasm accession TCGS-1862 against soil born and foliar diseases of groundnut

S. No.	Genotypes	Dry root rot (%)	Stem Rot (%)	ELS (1-9 scale)	LLS (1-9 scale)	Rust (1-9 scale)
1	TCGS-1862	0.0	0.0	2	1	1
	Checks					
1	National-JL24	21.3	16.8	9	7	7
2	Local-Narayani	17.6	14.2	8	7	6

Table 3: Reaction of groundnut germplasm accession TCGS-1862 against viral PSND/PBND diseases of groundnut

S. No.		Plant stand	PSND Plants (No.)	PSND (%)	PBND Plants (No.)	PBND (%)
1	TCGS-1862	121	6	9.9	4	6.3
1	National-JL24	68	28	36.7	15	22.0
2	Local-Narayani	59	19	32.2	14	23.7

leaf spot disease reduce the yield and also have an adverse affect on seed quality and grade characteristics, deteriorate the quality of plant biomass and thus render the fodder unsuitable as animal feed.

Hence, it is necessary to breed for high yielding genotypes with resistance/tolerance to collar rot and stem rot , late leaf spot diseases and tolerant to drought and sucking insects to increase and stabilize groundnut production.

Groundnut genotype TCGS 1862 was developed from KDG-128 x NRCG-CS-425 following the modified pedigree method of breeding and was found promising for the required attributes *viz.*, resistance to stem rot, collar rot, late leaf spot diseases , tolerant to drought, and sucking insects and defoliators. TCGS 1862 has recorded high pod and kernel yields.

Morpho-Agronomic Characteristics

The plants are medium stature (25-30 cm), leaflets are Small with dark green in colour. The stem is angular, Growth habit is erect to decumbent-3, sequential flowering and the branching pattern is sequential. The leaves remain green up to harvest (stay green character). It is resistance to stem rot, collar rot, late leaf spot diseases, tolerant to drought and sucking insects. Moderate pod constriction and moderate reticulation. Seeds have rose testa colour. It has recoded shelling out-turn of 62%

Reference

Mayee, C.D. and V.V. Datar. 1988. Diseases of groundnut in the tropics. Review Tropical Plant Pathology. **5:** 169-198.

46. ICS-200 (IC0638880; INGR21157), a castor (*Ricinus communis* L.) germplasm with resistance to leafhopper (*Empoasca flavescens*) and thrips (*Scirtothrips dorsalis*)

T Manjunatha*, AJ Pranhakaran, C Lavanya, P Duraimurugan, MS Lakshmi Prasad and Ramya KT

ICAR-Indian Institute of Oilseeds Research, Hyderabad-500030, Telangana, India

**Email: t.manjunatha@icar.gov.in*

Introduction

Castor (*Ricinus communis* L.), 2n=20, is an important commercial, industrial and non-edible oilseed crop suitable for both rainfed and irrigated cultivation in tropical and sub-tropical climatic conditions. India contributes for 85% of the castor production with highest productivity in the world. During 2019-20, castor was cultivated in an area of 9.70 lakh hectares with a production of 19.50 lakh tonnes and

productivity of 2010 kg/ha. Gujarat and Rajasthan account for 92% of the total castor area under irrigated conditions while 8% of the total castor area is under rainfed conditions (Solvent Extractors of India, India). The present genetic stock, ICS-200 was developed through conventional breeding technique *viz*., intra varietal hybridization (VP-1 x 48-1) followed by pedigree method of selection and generation advancement at ICAR-IIOR, Hyderabad.

Morpho-agronomic characteristics

Leafhopper, *Empoasca flavescens* (Cicadellidae: Homoptera) is the most important sucking insect pests in all the castor growing areas in India and causing severe hopper burn damage during both *kharif* and *rabi* seasons. Thrips, *Scirtothrips dorsalis* (Thripidae: Thysanoptera) feed on terminal leaves, floral parts and immature capsules results in leaf curling and drying of flowers and immature capsules. The proposed genotype, ICS-200 was screened at three hot spot locations *viz*., ICAR-IIOR-Hyderabad (Telangana), Palem (Telangana) and SK Nagar (Gujarat) consecutively for two years (2018-19 and 2019-20) using infester row method (in sandwich technique to increase leafhopper and thrips infestation) which was the standard screening method used in AICRP on Castor. Two years of screening under high leafhopper population confirmed the resistance of ICS-200 against leafhopper (hopper burn grade of 0 to 1 on 0–4 scale) at all the three locations in both the years of screening (Table 1) compared to susceptible check DPC-9 (hopper burn grade 3 to 4). Based on two years data, the monoecious line *viz*., ICS-200 was also found promising against thrips with low infestation of thrips on spikes (3.1 to 20.5 thrips/spike) at all the three locations as compared to susceptible check, DCS-9 (17.5 to 42.9 thrips/spike) (Table 2)

It is green, triple bloom and has non-spiny and non-dehiscent capsules. Other distinct features include short plant height (40-45 cm up to primary spike), loose spike, low number of nodes to the primary spike (mean 9.1), basal branching, early flowering of primary spike (40 days after sowing) and early maturity of primary spike (85-90 DAS). It has primary spike of length 38 to 41 cm, 100 seed weight of 24-26 grams with an oil content of 47-48%.

Table 1: Reaction of ICS-200 against leafhopper in multi-locations in different years

Genotype	*Year of screening*	*Hyderabad*	*Palem*	*SK Nagar*
		HPB (0-4 scale)	*HPB (0-4 scale)*	*HPB (0-4 scale)*
ICS-200		1	1	1
DPC-9 (SC)	2018-19	4	3	4
DCH-519 (RC)		1	1	2
ICS-200		1	0	1
DPC-9 (SC)	2019-20	4	3	4
DCH-519 (RC)		1	0	1

HPB – Hopper burn [0 – No injury (Highly Resistant); 1 – upto 10% (Resistant); 2 - 11 to 25% (Moderately Resistant); 3 -26 to 50% (Susceptible); 4 – above 50% (Highly Susceptible)], SC- Susceptible check; RC – Resistant check

Table 2: Reaction of ICS-200 against thrips in multi-locations in different years

	Year of Screening	*Thrips/spike*		
		Hyderabad	*Palem*	*SK Nagar*
ICS-200	2018-19	10.6	3.1	7.6
DCS-9 (SC)		42.9	17.5	20.8
M-574 (RC)		6.2	5.9	16.6
	CD (P=0.05)	11.1	2.1	1.5
ICS-200		13.0	9.3	20.5
DCS-9 (SC)	2019-20	39.8	29.3	32.2
M-574 (RC)		20.7	11.6	28.8
	CD (P=0.05)	10.3	5.0	2.2

SC- Susceptible check; RC – Resistant check

Associated characters and utility

It also has low node number making it early flowering and maturity and hence ICS-200 will be useful as a male parent to develop high yielding early castor hybrids and varieties with resistance to leafhopper and thrips.

47. DRMRHT-13-22-10 (IC640708; INGR21158), an Indian mustard (*Brassica juncea* L.) germplasm with heat tolerance at juvenile stage under field conditions

Bhagirath Ram*, VV Singh, Priyamedha, KH Singh, HK Sharma, MS Sujith Kumar, RS Jat, Pankaj Sharma, HP Meena and PK Rai

ICAR-Directorate of Rapeseed Mustard Research, Bharatpur-321001, Rajasthan, India

**Email: bhagirathram_icar@yahoo.com*

Brassica juncea is the predominant oilseed brassica in the Indian subcontinent. The growing of Indian mustard in Rajasthan is mostly carried out under conserve soil moisture conditions where sowing commences after south west monsoon rains. Early rains may lead the farmers to sow the crop early in the season to take advantage of conserved moisture in the soil. However, at the time of early sowing (second fortnight of September to first fortnight of October),

the mean surface soil temperature may reach as high as 45°C. High soil temperature often results in seedling mortality upon initial germination which may eventually require re-sowing. Heat stress at the seedling stage can increase mortality and has become an increasing threat for Indian mustard cultivation. Hence, this study was planned to evolve, evaluate and identify some of the promising heat stress tolerant lines from a pool of advanced breeding lines, to be used as probable donors for transferring heat tolerance.

DRMRHT-13-22-10a genotype of Indian mustard derived from crosses between JN032 X BPR549-9 and further selection were made through pedigree-selection method. Strain DRMRHT-13-22-10 was evaluated alongwith 7 advanced breeding lines and 2 checks *i.e.* NPJ- 112 and BPR 543-2 to assess the heat tolerance between advanced genotypes under heat stress conditions during rabi2017-18at the ICAR-DRMR, Bharatpur. The genotype DRMRHT-13-22- 10 recorded maximum relative water content (81.22%) as compared to best check NPJ112 (76.08%) for heat tolerance. DRMRHT-13-22-10genotype was evaluated alongwith 7 advanced breeding lines and 2 checks to assess the heat tolerance between advanced genotypes under heat stress conditions during 2018-19.The genotype DRMRHT-13-22-10 recorded maximum membrane stability index (23.02%) as compared to best check NPJ112 (16.06%). The genotype DRMRHT-13-22-10 recorded maximum water retention capacity of leaves (67.87%) as compared to best check NPJ112 (59.08%) for enhanced heat stress tolerance.

The proposed strain (DRMRHT-13-22-10) is heat tolerant at juvenile stage under field conditions based on testing under AICRP-RM trials *(Annual report 2019-20 pp phy 01, 04 and 2020-21 pp phy 05, 06)*. The genetic stock was tested under field conditions at four locations for two years. Based on pooled data over two years and locations revealed seedling mortality was 20.6% and dry wt./10 seedlings was 6.3g. Hence it was rated as heat tolerant at juvenile stage under field conditions. The proposed strain was tested for physiological traits at Bharatpur during 2017-18 and 2018-19 and it found to possess high MSI, RWC in comparison to checks.

48. Jor Lab KH-2 (IC640709; INGR21159), a black Zedoary/black turmeric (*Curcuma caesia* Roxb.) germplasm with the fresh weight rhizome essential oil content > 0.8%

Mohan Lal
CSIR-North East Institute of Science and Technology, Jorha-785006t, Assam, India
Email: drmohanlal80@gmail.com

Black turmeric (*Curcuma caesia* Roxb.) is an important medicinal plant belonging to zingiberaceae family. Although this plant is native to North East India, this is also found in Bangladesh as a wild species (Borah *et al*; 2019). The rhizomes of black turmeric have a high economical importance owing to its putative medicinal properties (Mahanta *et al*; 2019). Rhizome of this plant is claimed to be useful in treating several disease like piles, leprosy, bronchitis, asthma, cancer, epilepsy, fever wounds, impotency, fertility tooth ache and vomiting etc (Paw *et al*; 2020a). Presently *Curcuma caesia* is considered to be a threatened since natural habitat is destroying widely through several human activities such as over-exploitation of black turmeric for traditional medicine purposes, industrialization, urbanization, etc (Paw *et al*; 2020b; Paw *et al*., 2021).

Therefore, the need of the hour is to develop high essential oil yielding germplasm *Curcuma caesia* suited to India and to maintain the sustainable cultivation and germplasm of these high value medicinal plant species. In this investigation a total of 135 germplasm were collected from different region of India during 2016 and planted in RBD design at experimental farm of CSIR- NEIST, Jorhat, Assam. After two year of the evaluation trial one high rhizome essential oil rich germplasm was identified and named as "Jor Lab KH-2". This identified germplasm were again planted in multilocation trial at five different locations namely Jorhat, Lakhimijan, Imphal, Pasighat and Shillong of NE India during 2019. The identified germplasm was superior over the check variety. The identified germplasm have an average of 124 cm plant height, 49 cm leaf length, 26% dry rhizome recovery, 0.80 % essential oil in fresh rhizome weight basis and 26.18 tones rhizome yield tones/ha. The essential oil of this species has very high commercial values and contain antimicrobial, anti-inflammatory activities and very less genotoxicity (Paw *et al* 2021).

References

Borah A, Paw M, Gogoi R, Loying R, Sarma N, Munda S,Pandey S. K and Lal M. (2019).Chemical composition, antioxidant, anti-inflammatory, anti-microbial and in-vitro cytotoxic efficacy of essential oil of *Curcuma caesia* Roxb. leaves: An endangered medicinal plant of North East India. *Ind.Crop. Prod*, **129:** 448-454.

Mahanta BP, Sut D, Kemprai P, Paw M, Lal M and Haldar S (2019). A ^{1}H-NMR spectroscopic method for the analysis of thermolabile chemical markers from the essential oil of black turmeric (*Curcuma caesia*) rhizome: application in post-harvest analysis. *Phytochem Anal*, 1-9 *doi:10.1002/pca.2863.*

Paw M, Gogoi R, Sarma N, Pandey SK, Borah A, Begum T and

Lal M. (2020a).Study of anti- oxidant, anti-inflammatory, genotoxicity, antimicrobial activities and analysis of different constituents found in rhizome essential oil of *Curcuma caesia* Roxb. collected from North East India. *Curr Pharm Biotechnol*, **21(5):** 403-413, *doi: 10.2174/1389201020666191118121609*.

Paw M, Munda, S, Borah A, Pandey SK and Lal M. (2020b). Estimation of variability, genetic divergence, correlation studies of *Curcuma caesia* Roxb. *JARMAP*, **17:**100-251

Paw M, Borah A, Pandey SK, Baruah J, Begum T and Lal M (2021). Simple sequence repeat marker based genetic diversity assessment amongst high essential oil yielding lines of *Curcuma caesia* Roxb. *Genet Resour Crop Evol*, **68:** 1345-1358.

49. Jor Lab CZ-6 (IC640710; INGR21160), a Narkachur (*Curcuma zeodaria*) germplasm with fresh weight rhizome essential oil content > 0.6%

Mohan Lal

CSIR-North East Institute of Science and Technology, Jorhat-785006, Assam, India

Email: drmohanlal80@gmail.com

Curcuma zedoaria has long been used as a folk medicine in India. Its smell is similar to that of turmeric and mango. The perennial herb has a warm-spicy, woody and camphoraceous cineolic odor and bears yellow shiny flowers, with red and green bracts. The plant is native to North East India and Indonesia. However, it is widely used as a spice in the Western counties. It is also found in sub-tropical regions of eastern Nepal. It is mainly found in eastern Himalayan. Major chemicals compounds are (+)- germacrone-4, 1,8-cineole, 5-epoxide, germacrone, furanodienone, curzerenone, zederone, dehydrocurdione, curcumenol, isocurcumenol, curcumenone, curmanolide A and curmanolide B found in the rhizome essential oil. Traditionally, the dried rhizome of *Curcuma zedoaria* is selected to make drinks or to be extracted as medicine (Mau *et al.,* 2003). The uses of *Curcuma zedoaria* in traditional medicine have been well documented (Prajapati *et al*; 2003). Rhizomes are used as a carminative, digestive stimulant, and in treatment of colds and infections. They exhibit both antibacterial and antifungal activity (Anonymous, 1985; Mau *et al.,* 2003). The essential oils from *Curcuma zedoaria*, obtained by steam distillation of dried tubers form an active ingredients in antibacterial preparations. Starches extracted from this species are used in diets for infants and convalescents due to their cooling and demulcent properties (Prajapati *et al*., 2003). It has been reported that the boiling water extracts of *Curcuma zedoaria* had a moderate antimutagenic activity against benzo[a] pyrene (Jeng *et al*., 2003).

Therefore the need of the hour is to develop essential oil yielding germplasm of *Curcuma zedoria* suited to India and to maintain the sustainable cultivation and germplasm of these high value medicinal plant species. In this investigation a total of 86 germplasm were collected from different region of India during

2016 and planted in RBD design at CSIR-NEIST experimental farm, Jorhat, Assam. After two year of the evaluation trial one high essential oil rich germplasm was identified and named as "Jor Lab CZ-6". This identified germplasm was again planted in multilocation trial at five different locations namely Jorhat, Lakhamijan, Imphal, Pasighat and Shillong of NE India during 2019. The identified germplasm was superior over the check variety. The identified germplasm having average of 144 cm plant height, 28 cm leaf length, 23% dry rhizome recovery, 0.65% essential oil in fresh rhizome weight basis and had an average rhizome yield of 31.01 t/ha. The characteristic of the selected germplasm was found to be stable for commercial cultivation.

References

Mau JL, Eric YC, Lai, Nai-Phon Wang, Chien-Chou Chen, Chi-Huarng Chang and Charng- Cherng Chyau (2003). Composition and antioxidant activity of the essential oil from *Curcuma zedoaria*. Food Chemistry, **82:** 583–591

Prajapati ND, Purohit SS, Sharma AK and Kumar T (2003). A Handbook of Medicinal Plants. Agrobios, India, p. 181.

Anonymous (1985). The Wealth of India—Raw Materials IA (Revised): A Dictionary of Indian Raw Materials and Industrial Products, vol. 69. CSIR, New Delhi, pp. 401–406

Jeng-Leun Mau, Eric YC Lai Nai-Phon Wang, Chien-Chou Chen, Chi-Huarng Chang and Charng-Cherng Chyau (2003). Composition and antioxidant activity of the essential oil from *Curcuma zedoaria*. *Food Chemistry*, **82:** 583-591

50. Jor Lab SK-154 (IC0633577; INGR21161), a nightshades (*Solanum khasianum* (C.B. Clarke) germplasm with white colour berries

Mohan Lal

CSIR-North East Institute of Science and Technology, Jorhat-785006, Assam, India

Email: drmohanlal80@gmail.com

One of the ethnic medicinal plants, *Solanum khasianum* (C.B. Clarke) also known as *Solanum aculeatissimum* Jacq., (2n=24) belongs to the family *Solanaceae* and grows wild in Meghalaya hills, Assam, Manipur, Sikkim, West Bengal, Orissa, Arunachal Pradesh and Nilgiri hills of India ascending to an altitude of 1600 m. In India, 33 species of *Solanum* are available (Maiti *et al.,* 1965). The present cultivation area of the species is more than 4000 ha in Maharashtra, Karnataka, Assam, Tripura, Meghalaya and Manipur state of India (Gogoi *et al.,* 2021). The presence of solasodine in the mature berries has a tremendous commercial possibility and completely yellowish berries show the presence of alkaloids in maximum quantity (Mann, 1978). Solasodine is employed as a hormone precursor within the steroid drug business for the manufacturing corticosteroids, anabolic steroids, antifertility drugs etc., in addition to its various medicinal properties (Kumar *et al*., 2019). It is a stout, branched, woody shrub attaining a height of 0.75 to 1.5 m. Stems are spiny, leaves are ovate to lobed bearing spines on both surfaces, the flowers are hermaphrodite. In general, the berries are yellowish when ripe, seeds are small brown in color and abundant (Gogoi *et al.,* 2021). A total of 273 accessions of *Solanum khasianum* were collected out of which 186 accessions rich in solasodine content (>0.8 %) were selected and planted in RBD design during the year 2017 which were again planted during 2018. After two year of evaluation, a germplasm bearing white berries was identified which was confirmed by planting it as multi-location trial, along with the standard check variety during the year 2019 at four different locations (Jorhat, Bokakhat, Pasighat and Madang) of Northeast India. The identified germplasm of *Solanum khasianum* which bears berries which are white in colour on maturity was found to possess an average solasodine content of 0.99% and average fresh berries yield of 4.2 t/ha. The identified trait was found to be stable for commercial cultivation and could be used in breeding program.

References

Gogoi R, Sarma N, Pandey SK and Lal M (2021). Phytochemical constituents and pharmacological potential of *Solanum khasianum* C.B. Clarke., extracts: Special emphasis on its skin whitening, anti-diabetic, acetylcholinesterase and genotoxic activities. *Trends Phytochem. Res*, **5(2):**47-61.

Kumar R, Khan MI, Prasad M and Badruddeen (2019). Solasodine: A Perspective on their roles in Health and Disease. *Res. J. Pharm. Technol*. 12(5), 2571-2576.

Maiti PC, Mookerjea S and Mathew R (1965). Solasodine from *Solanum khasianum*. US department of Agriculture. **54:** 1828-1829.

Mann JD (1978). Production of solasodine for the pharmaceutical industry. *Adv. Agron*, **30:** 209-215.

51. Jor Lab SK-3 (IC0633426; INGR21162), a nightshade (*Solanum khasianum* (C.B. Clarke)) germplasm with high fruit solasodine content

Mohan Lal

CSIR-North East Institute of Science and Technology, Jorhat-785006, Assam, India

Email: drmohanlal80@gmail.com

One of the ethnic medicinal plants, *Solanum khasianum* (C.B. Clarke) also known as *Solanum aculeatissimum* Jacq., (2n=24) belongs to the family *Solanaceae* and grows wild in Meghalaya hills, Assam, Manipur, Sikkim, West Bengal, Orissa, Arunachal Pradesh and Nilgiri hills of India ascending to an altitude of 1600 m. 33 species of *Solanum* are available In India (Maiti *et al*., 1965). The present cultivation area of the species is more than 4000 ha in Maharashtra, Karnataka, Assam, Tripura, Meghalaya and Manipur state of India (Gogoi *et al*; 2021). The presence of phytochemical compounds in the plant indicates their medicinal potential which can be determined by phytochemical analysis (Devi *et al*., 2014). Such knowledge of plants chemical constituents is desirable due to the presence of great economically and medicinally important substances which can be used as drugs (Chetri *et al*; 2008). The presence of solasodine in the mature berries has a tremendous commercial possibility and completely yellowish berries show the presence of alkaloids in maximum quantity (Mann, 1978). Solasodine is employed as a hormone precursor within the steroid drug business for the manufacturing corticosteroids, anabolic steroids, antifertility drugs etc. Isolated solasodine from solanum plants exploits its medicinal properties such as anticonvulsant, CNS depressant, antioxidant, cytotoxic, antinociceptive, anti-inflammatory, hepatoprotective, immunomodulatory, antiatherosclerotic, antimicrobial, and antiobesity activity, etc (Kumar *et al*., 2019). Owing to its multiple application of solasodine, the identification of high solasodine content germplasm would be highly beneficial for pharmaceutical and commercial purposes. A total of 273 accessions of *Solanum khasianum* were collected out of which 186 accessions rich in solasodine content (>0.8 %) were selected and planted in RBD design during the year 2017 which were again planted during 2018. After two year of evaluation, a high solasodine-rich line Jor Lab SK-3 was identified which was confirmed by planting in multi-location trial, along with the standard check variety during the year 2019 at four different locations (Jorhat, Bokakhat, Pasighat and Madang) of Northeast India. Jor Lab SK-3 had an average solasodine content of 1.30%. Jor Lab SK-3 was found to be

superior in solasodine content trait and was found to be stable for commercial cultivation and could be used in the breeding program.

References

Chetri HP, Yogol NS, Sherchan J, Anupa K.C, Mansoor S and Thappa P (2008). Phytochemical and antimicrobial evaluations of some medicinal plants of Nepal Kathmandu University. K.U.S.E.T, **1:** 49-54.

Devi KR, Subramani V, Nakulan VR and Annamalai P (2014). Qualitative and quantitative phytochemical analysis in four pteridophytes. *Int. J. Pharm. Sci. Res***, 27:** 408-412.

Gogoi R, Sarma N, Pandey SK and Lal M (2021). Phytochemical constituents and pharmacological potential of *Solanum khasianum* C.B. Clarke., extracts: Special emphasis on its skin whitening, anti-diabetic, acetylcholinesterase and genotoxic activities. *Trends Phytochem. Res,* **5(2):** 47-61.

Kumar R, Khan MI, Prasad M and Badruddeen (2019). Solasodine: A Perspective on their roles in Health and Disease. *Res. J. Pharm. Technol*, **12(5):** 2571-2576.

Maiti PC, Mookerjea S and Mathew R (1965). Solasodine from *Solanum khasianum*. *US department of Agriculture*, **54:** 1828-1829.

Mann JD (1978). Production of solasodine for the pharmaceutical industry. *Adv. Agron*, **30:**209-215.

52. JOR LAB SK-124 (IC0633547; INGR21163), a nightshade (*Solanum khasianum* C.B. Clarke) germplasm with thornless leaves and stem.

Mohan Lal

CSIR-North East Institute of Science and Technology, Jorhat-785006, Assam, India

Email: drmohanlal80@gmail.com

Solanum khasianum C.B. Clarke, also known as *Solanum aculeatissimum* Jacq., belongs to the family *Solanaceae* and grows wild in Meghalaya hills, Assam, Manipur, Sikkim, West Bengal, Orissa, Arunachal Pradesh and Nilgiri hills of India ascending to an altitude of 1600 m. In India, 33 species of *Solanum* are available (Maiti *et al.,* 1965). The present cultivation area of the species is more than 4000 ha in Maharashtra, Karnataka, Assam, Tripura, Meghalaya and Manipur state of India (Gogoi *et al.,* 2021). The presence of solasodine in the mature berries has a tremendous commercial possibility and completely yellowish berries show the presence of alkaloids in maximum quantity (Mann, 1978). Solasodine is employed as a hormone precursor within the steroid drug business for the manufacturing corticosteroids, anabolic steroids, antifertility drugs etc. Isolated solasodine from solanum plants exploits its medicinal properties such as anticonvulsant, CNS depressant, antioxidant, cytotoxic, antinociceptive, anti-inflammatory, hepatoprotective, immunomodulatory, antiatherosclerotic, antimicrobial, and antiobesity activity, *etc* (*Kumar et al.,* 2019). The plant is a stout, branched, woody shrub attaining a height of 0.75 to 1.5 m. Stems are spiny, leaves are ovate to lobed bearing spines on both surfaces, the flowers are hermaphrodite, the berries are yellowish when ripe, seeds are small brown in color and abundant. Being a cheaper source for solasodine the berries are in high demand. However, the presence of spiny thorns on the leaves makes the harvesting a tedious process as mechanical harvesting is not possible in the plant. In this investigation, a total of 273 accessions of *Solanum khasianum* were collected out of which 186 accessions rich in solasodine content (>0.8 %) were selected and planted in RBD design during the year 2017 which were again planted during 2018. After two years of evaluation, JOR LAB SK-124 a thorn-less germplasm of *Solanum khasianum* was identified, which was further confirmed by planting it as multi-location trial, along with the standard check variety during the year 2019 at four different locations (Jorhat, Bokakhat, Pasighat and Madang) of Northeast India. JOR LAB SK-124 in which thorns are absent on the leaves would be highly efficient for harvesting of the berries. The germplasm was found to have average fresh berries yield of 5.5 t/ha with an average solasodine content of 1.10%. Furthermore, the identified trait was found to be stable in multilocation testing and the germplasm could be a source of unique traits.

References

Gogoi R, Sarma N, Pandey SK and Lal M (2021). Phytochemical constituents and pharmacological potential of *Solanum khasianum* C.B. Clarke., extracts: Special emphasis on its skin whitening, anti-diabetic, acetylcholinesterase and genotoxic activities. *Trends Phytochem. Res*, **5(2):** 47-61.

Kumar R, Khan MI, Prasad M and Badruddeen (2019). Solasodine: A Perspective on their roles in Health and Disease. *Res. J. Pharm. Technol*, **12(5):** 2571-2576.

Maiti PC, Mookerjea S and Mathew R, (1965). Solasodine from *Solanum khasianum*. *US*

department of Agriculture. **54:** 1828-1829.

Mann JD (1978). Production of solasodine for the pharmaceutical industry. *Adv. Agron*, **30:** 209- 215.

53. JOR LAB SK-9 (IC0633432; INGR21164), a nightshade (*Solanum khasianum* C.B. Clarke.) germplasm with red colour berries at ripening stage

Mohan Lal
CSIR-North East Institute of Science and Technology, Jorhat-785006, Assam, India
Email: drmohanlal80@gmail.com

Solanum khasianum C.B. Clarke, belonging to the family *Solanaceae* grows wild in Meghalaya hills, Assam, Manipur, Sikkim, West Bengal, Orissa, Arunachal Pradesh and Nilgiri hills of India ascending to an altitude of 1600 m. In India, 33 species of *Solanum* are available (Maiti *et al*., 1965). The present cultivation area of the species is more than 4000 ha in Maharashtra, Karnataka, Assam, Tripura, Meghalaya and Manipur state of India (Gogoi *et al*., 2021). The plant is a rich source for solasodine. The presence of solasodine in the mature berries has a tremendous commercial possibility and completely yellowish berries show the presence of alkaloids in maximum quantity (Mann, 1978). Solasodine is employed as a hormone precursor within the steroid drug business for the manufacturing corticosteroids, anabolic steroids, antifertility drugs etc. Isolated solasodine from solanum plants exploits its medicinal properties such as anticonvulsant, CNS depressant, antioxidant, cytotoxic, antinociceptive, anti- inflammatory, hepatoprotective, immunomodulatory, antiatherosclerotic, antimicrobial, and antiobesity activity, etc (Kumar *et al*., 2019). It is a stout, branched, woody shrub attaining a height of 0.75 to 1.5 m. Stems are spiny, leaves are ovate to lobed bearing spines on both surfaces, the flowers are hermaphrodite. In general, the berries are yellowish when ripe; seeds are small brown in color and abundant (Gogoi *et al*., 2021). A total of 273 accessions of *Solanum khasianum* were collected out of which 186 accessions rich in solasodine content (>0.8%) were selected and planted in RBD design during the year 2017 which were again planted during 2018. After two years of evaluation, a germplasm JOR LAB SK-9 was identified bearing red berries which was confirmed by planting it as multi-location trial, along with the standard check variety during the year 2019 at four different locations (Jorhat, Bokakhat, Pasighat and Madang) of Northeast India. JOR LAB SK-9 bears berries which are red in colour and turns deep red on maturity was found to be stable with an average solasodine content of 1.01% and average fresh berries yield of 3.5 t/ha. The plant with the trait of berries red in colour was found stable for commercial cultivation and could be used in the breeding program.

References

Gogoi R, Sarma N, Pandey SK and Lal M (2021). Phytochemical constituents and pharmacological potential of *Solanum khasianum* C.B. Clarke., extracts: Special emphasis on its skin whitening, anti-diabetic, acetylcholinesterase and genotoxic activities. *Trends Phytochem. Res*, **5(2):** 47-61.

Kumar R, Khan MI, Prasad M and Badruddeen (2019). Solasodine: A Perspective on their roles in Health and Disease. *Res. J. Pharm. Technol*, **12(5):** 2571-2576.

Maiti PC, Mookerjea S and Mathew R (1965). Solasodine from *Solanum khasianum*. US department of Agriculture. **54:** 1828-1829.

Mann JD (1978). Production of solasodine for the pharmaceutical industry. *Adv. Agron,* 30: 209-215.

54. JOR LAB ZB-103 (IC640711; INGR21165), a ginger [*Zingiber zerumbet* (L.) Roscoe ex Sm.] germplasm with high fresh weight of rhizome and high content of essential oil

Mohan Lal
CSIR-North East Institute of Science and Technology, Jorhat-785006, Assam, India
Email: drmohanlal80@gmail.com

Abstract

Zingiber zerumbet (L.) Roscoe ex Sm. belonging to the Zingiberaceae family is an aromatic herbal species. This species is commonly known as Asian ginger, bitter ginger, shampoo ginger. It is a perennial tuberous herb plant commonly found in damp areas and areas with partial shade (Yob *et al*; 2011). It is cultivated widely in South East Asia countries, tropical and subtropical regions around the world (Rashid *et al*., 2005; Baby *et al*., 2009).

Zingiber zerumbet of the Zingiberaceae family is known as wild edible ginger having immense medicinal properties (Al-Zubari *et al* 2010 ; Eid *et al* 2011). The present study was aimed to identification of high essential oil yielding genotypes tested through stability parameters at multilocation trials. During the year 2017 a total of 47 genotypes of *Z. zerumbet* was collected and planted at CSIR-NEIST experimental farm. A selection trial was conducted for two years *viz.,* 2017 and 2018 for all the collected germplasm which led to identification of JOR LAB ZB-103 with high fresh rhizome and essential oil yielding traits. JOR LAB ZB-103 was subjected to stability analysis along with two check varieties at five multilocation trials at five different locations

namely Jorhat, Lakhamijan, Imphal, Pasighat and Shillong of NE India in March, 2019. JOR LAB ZB-103 and an average fresh rhizome yield of 24.86 t/ha and an average of 0.75% essential oil yield on fresh weight basis. The assessment of stable performance was evaluated using Eberhart and Russell model. Variable environments were found to be present in the study as revealed by the significant variance due to environment and environment (linear). The genotype × environment interaction of the selected genotype also showed significant variance for all the traits revealing the well interaction of the genotype with all the environmental conditions. On comparative assessment, the identified genotype was found to be highly stable with high adaptability to varying environments for all the traits under studied. While both the check varieties revealed low adaptability to varying environments for the higher-yielding traits. The GC/MS analysis of the essential oil of Jor Lab ZB-103 revealed zerumbone (32.79%) as the major compound followed by camphene (19.41%) and eucalyptol (6.8%). The other compounds were present as minor and trace compounds. Thus, the high yielding genotype Jor Lab ZB-103 was found to be highly stable genotype which could be made available in the public domain for wide-scale cultivation.

Table 1: Estimation of stability ameters for six traits of selected genotype and check varieties at multilocation trials

Genotype	*Plant height (cm)*		*No. of tillers/ plant*		*Leaf length (cm)*		*Dry rhizome recovery %*		*Fresh rhizome yield (t/ha)*		*Essential Oil %*							
	βi	σ^2di	βi	σ^2di	βi	σ^2di	βi	σ^2di	βi	σ^2di	βi	σ^2di	βi	σ^2di	βi	σ^2di	βi	σ^2di
Jor Lab	1.013	0.227	0.105	0.248	0.794	0.634	0.265	0.675	0.695	0.078	-	-						
ZB-103	3	7	9	5	7	7	9	4	9	8	0.96	0.000						
											0	1						
Changla	1.219	0.372	1.055	0.985	-	0.807	0.481	1.425	0.949	0.528	1.83	0.000						
ng local	7	1		9	0.252	4	5	4	2	0	0	0						
					5													
RRLJZB	0.767	0.024	1.447	0.506	0.457	0.205	2.037	0.721	1.354	0.633	2.13	0.000						
-151	0	5	1	5	8	9	0	7	9	0	0	1						

βi = regression coefficient, σ^2di = deviation from regression

References

Yob NJ, Jofrry SM, Affandi MMRMM, The LK, Salleh MZ and Zakaria ZA (2011). *Zingiber zerumbet* (L.) Smith: a review of its ethnomedicinal, chemical, and pharmacological uses. Evidence-Based Compl. *Alt. Med*, 2011: 543216.

Rashid RA and Pihie AHL (2005). The antiproliferative effect of *Zingiber zerumbet*

extract, *Malaysian J Pharmaceut. Sci*, **3:** 45–52.

Baby S, Dan M, Thaha ARM, Johnson AJ, Kurup R, Balakrishnapillai P and Lim CK (2009). High content of zerumbone in volatile oils of *Zingiber zerumbet* from southern India and Malaysia. *Flavour Frag. J*, **24:** 301-308.

Al-Zubairi AS, Abdul AB, Yousif M, Abdelwahab SI, Elhassan MM and Mohan S (2010). In vivo and in vitro genotoxic effects of zerumbone. *Caryologia,* **63:** 11–17.

Eid EEM, Abdul AB, Suliman FEO, Sukari MA, Rasedee A and Fatah SS (2011). Characterization of the inclusion complex of zerumbone with hydroxypropyl- cyclodextrin. *Carbohydr. Polym*, **83:** 1707–1714.

55. IIHRJ3-2 (IC0624508; INGR21166), a china aster [*Callistephus chinensis* (L.) Nees.] germplasm with Red Purple flower colour group, long flower stalk and long vase life

Rajiv Kumar*, T Manjunatha Rao and T Usha Bharathi

ICAR-Indian Institute of Horticultural Research, Bengaluru-560089, Karnataka, India

**Email: Rajiv.Kumar11@icar.gov.in*

Introduction

China aster is commercially important popular annual flowering plant belonging to the family Asteraceae. In India, it is grown traditionally for loose flower, cut flower, landscape, floral decoration, making garlands and *venis* (Rao *et al*., 2012). The China aster germplasm IIHRJ3-2 is

derived from the cross Arka Kamini x Local White following pedigree selection and it was developed at ICAR- Indian Institute of Horticultural Research, Bengaluru, Karnataka (13° 58' N Latitude, 78°E Longitude and at an altitude of 890 meter above mean sea level), India. The germplasm IIHRJ3-2 has unique semi- double, very light pink flower colour (Red Purple group, 65D, Fan 2) with long flower stalk and vase life.

Table 1: Morpho-agronomic description of China aster germplasm IIHRJ3-2

Sl. No.	*Traits Description*	*Average value*
1.	Plant height (cm)	67.89
2.	Plant spread (cm)	26.00
3.	Days to flower	73.33
4.	Number of branches per plant	10.33
5.	Flower head diameter (cm)	5.04
6.	Number of flowers per plant	56.44
7.	Stalk length (cm)	47.67
8.	Vase life (days)	10.11
9.	Flower colour as per RHS Colour Chart	Red Purple group, 65D, Fan 2
10.	Flower form	Semi-double
11.	Utility	Cut flower and flower arrangement

Morpho-Agronomic Characteristics

The China aster germplasm IIHRJ3-2 has erect plants with average plant height (67.89 cm), number of branches per plant (10.33) and number of flowers per plant (56.44). Its flowers has unique flower colour (Red Purple group, 65D, Fan 2).

Associated Characters and Cultivated Practices

The flowers of China aster germplasm IIHRJ3-2 is having very light pink flower colour (Red Purple group, 65D, Fan 2), semi-double flower type with 5 to 6 whorls of ray florets. It has average plant spread (26.00 cm), days to flower (73.33 days), flower head diameter (5.04 cm), stalk length (47.67) and vase life (10.11 days). It is found suitable for cut flower and flower arrangement.

It grows best in open and well drained loamy soil with soil pH 6 to 7. A temperature of 20° to 30°C during day and 15° to 17°C during night with relative humidity of 50-60% is most suitable for its growth and flowering. Thirty days seedlings are transplanted at a spacing of 30 cm x 30 cm. Plants are pinched 35 to 40 days after transplanting. The China aster is extensively grown in Karnataka, Tamil Nadu, West Bengal and Maharashtra. The germplasm IIHR3-2 is found suitable for cut flower and flower arrangement.

Reference

Rao TM, Rajiv Kumar and PB Gaddagimath (2012). China aster. *Extension Bulletin*. The Director. ICAR-IIHR, Bengaluru, pp 1-16.

56. CZC-94 (IC0640712; INGR21167), a Cumin (*Cuminum cyminum* L.) germplasm with early flowering and early plant maturity under normal condition of sowing

Rajesh Kumar Kakani* **and Ramesh Kumar Solanki**

ICAR-Central Arid Zone Research Institute, Jodhpur-342003, Rajasthan, India

**Email: rajesh.kakani@icar.gov.in*

Cumin (*Cuminum cyminum*) is the important commercial crop of western arid regions of India. The available diversity in cumin germplasm is low for agro-morphological traits and all cultivated varieties are of longer duration (125-135 days). Among the cultivars, Gujarat Cumin-4 (GC-4), is most popular in arid regions of India. In arid regions the cost of cultivation for raising a long duration crop is high considering the requirement of irrigation water and plant protection. Hence, early maturing cumin cultivar of 100 to 110 days duration has been a felt need for resources limited arid regions.

At ICAR-CAZRI, Jodhpur an early maturing natural variant was selected in *Rabi* 2017-18 from seed production plot of the GC-4; denominated as CAZRI Cumin-94 (CZC-94). Genotype CZC-94 commence flowering in around 40 days and matured in less than 100 days; whereas its parent population (GC-4) flowers in 65-70 days and

Table 1: Phenotypic expression of GC-4 and four morphotypes selected during rabi 2017

S. No	*Marker traits*	*GC-4*	*MT-1*	*MT-2*	*MT-3*	*MT-4*
1	FID	61-65	42	65	63	62
2	FC	Pink (P)	Pink (P)	White (W)	White (W)	Pink (P)
3	PT	Bushy with high biomass (BHB)	Open canopy with low biomass (OLB)	Bushy withhigh biomass (BHB)	Erect (E)	Erect with high biomass (EHB)
4	Plant height	35-40 cm	25 cm	32 cm	40 cm	45 cm

FID: Flowering initiation in days; FC: Flower colour; PT: plant type

Table 2: Phenotypic expression of the morpho-types during year 2017, 2018 and 2019

Marker Traits /Year	*MT-1*	*MT-2*	*MT-3*	*MT-4*								
	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
FID	42	40-43	42-44	65	64-68	62-66	63	61-65	62-65	58	55-60	59-62
FC	P	P	P	W	W	W	W	W	W	P	P	P
PT	OLB	OLB	OLB	BHB	BHB	BHB	E	E	E	EHB	EHB	EHB

FID: Flowering initiation in days; FC: Flower colour; PT: plant type

Table 3: Phenotypic expression for marker traits observed over number of plants over years

20192017Morpho- typesS.No
NPNENPEPSSSNPNENPEPSSSNP
0325325200075755001MT 11
0340340200080805001MT 22
0328328200078785001MT 33
0335335200085855001MT 44

NP: Number of plants; SS: No of seeds sown; PS: Plant stand at observation; NPE: No of plants expressing the marker trait; NPNE: No of plants not expressing the marker trait

matures in 130-135 days. Subsequently in 2018-19, CZC-94 was evaluated in 'plant to row progeny' for heritability of earliness and interestingly all plants showed the same expression for flowering and maturity. The bulk harvest of CZC-94 was further evaluated in *Rabi* 2019-20 with popular released varieties *viz.,* GC-4, RZ-19, RZ-209 and RZ-223 under variable date of sowings. Again, same performance for flowering i.e. 40-42 days was observed in all dates of sowings demonstrated high heritability of earliness. This much needed early maturity cumin genotype has high prospects to be developed as cultivar for the resource limited cumin growing regions of the country.

57. IC0635379 (IC0635379; INGR21168), a Jamun [*Syzygium cuminii* (L.) Skeels] germplasm with seedless character

Prakash Chandra Tripathi*, A Rekha, Anuradha Sane, VK Rao and M Arivalgan

ICAR-Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru- 560089, Karnataka, India

**Email: prakaashtripathii2000@yahoo.co.in*

Jamun (*Syzygium cuminii* (L.) Skeels) is an evergreen tree of tropical and subtropical region. It is originated from Indian subcontinent. Jamun, widely known as Jambuphalam in Sanskrit, is extensively used in several holistic treatments of Ayurveda, Unani, Siddha and Chinese medicines. Jamun is found grown as a wild and semi-wild tree in entire tropical and subtropical parts of India. Jamun cultivation is gaining popularity in the last decade with release of new varieties and increased demand for various reasons. Vast genetic and species diversity exists in the jamun in India. Sizable diversity of jamun is observed in Maharashtra, Rajasthan, Gujarat, Uttar Pradesh, Haryana, West Bengal, and the Western Ghats region. (Daware *et al.,* 1985; Malik *et al.*, 2017). Selection is the crop improvement method widely adopted in jamun and several varieties are being released using this method (Keskar *et al,* 1989). Extensive surveys which were undertaken in different parts of the country lead to several collections of jamun. These collections were planted in the field at ICAR-Indian Institute of Horticulture Research, Bengaluru and *ex- situ* evaluation at ICAR-Indian Institute of Horticulture Research, Bengaluru.

A seedless jamun collection was identified from the collections made earlier from Western Ghats. The collection does not have any seed and not even rudimentary seed is present and the whole fruit is edible. The tree is spreading type. The leaf length is 10.53 cm while the leaf width is 6.30cm. The petiole length is 2.52 cm. It flowers during March -April, almost 15-20 days later than other jamun genotypes an d the fruit matures in the month of July. The fruits are available almost 15-20 days later as compared to Cv. Dhoopdal under Bengaluru conditions. The fruits are produced in clusters of up to 15 fruits. The tree yield more than 10000 fruits (10-15 kg) per tree. The fruits are oblong shape and weighing about 0.8 to 1.3 g. The fruit colour is dark purple and pulp is pinkish white (Table 1). The juice content is 62.2 percent and total soluble solids are 13.5^{O} Brix. capacity is 726.2 mg and 1391 mg AE/100 g, as measured in terms of DPPH radical scavenging activity and FRAP reducing power, respectively. The *anthocyanin* content (230mg/100g) is higher than the Dhoopdal variety (124mg/100g). The total phenols are 10.25 mg/g and total flavonoids are 227 mg (Table 1). There is no seed and only one pink spot is visible in the transverse section of the fruit.The selection can be used as fresh as well as processing. This can also be used in breeding programme for decreasing seed size and incorporation of selflessness.

Table 1: Comparison of Growth and fruit characters of seedless selection with Dhoopdal

Traits	*Seedless selection*	*Dhoopdal*	*Traits*	*Seedless selection*	*Dhoopdal*
IC number	IC-0635379	IC-0621955	Fruit weight (g)	1.1	10.52
Tree growth	Spreading	Spreading	Fruit length (cm)	1.37	3.16
Leaf length (cm)	10.53	11.32	Fruit width (cm)	0.63	2.01
Leaf breadth (cm)	6.30	6.82	Fruit shape	Oblong	Oblong
Petiole length (cm)	2.52	1.91	Fruit colour	Dark Purple	Dark purple
Flowering time	March -April	Feb- March	Juiciness	High	Medium
Bearing patten	Clusters up 15, interior bearing	Clusters up 6-8, interior bearing	Fruit taste	Sweet	Sweet
Maturity period	July	June	Pulp colour	Pinkish White	Whitish purple
Yield (No. of fruits)	> 10000	> 3000	Pulp weight (g)	1.1	7.04
Yield (kg)	8-10kg	40-50 kg	Pulp-seed ratio	-	4.76
TSS (°Brix)	13.5	17.33	Anthocyanins(mg/100 g FW Pulp)	230	124
Acidity (%)	0.17	0.21	Total phenols mg/100 g FW Pulp	1025	335
Total Sugar (%)	6.72	6.35	DPPH mg AE / 100 g FW Pulp	726.1	403
Reducing sugar	3.53	3.25	FRAP Antioxidant activity mgAE/ 100 g FW Pulp	1391	316

References

Daware SG, VR Chakrawar and ST Borkar (1985). Variability and correlation studies in Jamun. *Punjab Hort*, **25 (1-4):** 89-93.

Malik SK, Rekha Chaudhury, Vartika Srivastava and Sanjay Singh (2017). Genetic Resources of *Syzygium cumini* in India-Present Status and Management, in the Genus *Syzygium. CRC Press*, pp 20, ISBN9781315118772.

Keskar G, AR Karale, BC Dhawale and KG Chouhari (1989). Improvement of jamun by selection. *Maharashtra J Hort*, **4:** 117-120.

58. MSH/14-7 (IC640713; INGR21169), an interspecific somatic hybrid-derived clone Potato (*Solanum tuberosum* L.) germplasm with wider genetic base, high yield combined with moderate resistance to late blight

Jagesh Kumar Tiwari[1*], Satish K Luthra[2], Vinod Kumar[1], Dalamu[1], Mehi Lal[2], Rajesh K Singh[1], Tanuja Buckseth[1], Vinay Bhardwaj[1] and Manoj Kumar[1]

[1]*ICAR-Central Potato Research Institute, Shimla-171001, Himachal Pradesh, India*

[2]*ICAR-CPRI Regional Station, Modipuram Campus, Meerut-250110, Uttar Pradesh, India*

[*]*Email: jageshtiwari@gmail.com*

Interspecific potato hybrid MSH/14-7 is a back-cross progeny of advanced stage clonal selection at ninth generation (BC_1-C_9). MSH/14-7 was developed by crossing between Kufri Garima and bulk pollen of somatic hybrids clones P1 to P14. These interspecific somatic hybrids were developed by protoplast fusion between *Solanum tuberosum* dihaploid 'C-13' and diploid wild potato species *S. pinnatisectum*. MSH/14-7 possesses wider genetic base in cultivated (*S. tuberosum*) from wild species (*S. pinnatisectum*) with high tuber yield (avg.44.72 tonnes/hectare), attractive tubers, shallow eyes depth, 20% tuber dry matter content, very good keeping quality and moderate resistance to late blight disease under the natural (hot-spot) field conditions at Kufri, Shimla. This shows successful utilization of somatic hybrids in developing a promising advanced stage hybrid (MSH/14-7) for high yield combined with late blight resistance to be used as a parental line in potato breeding in future. Additionally, molecular profiling using SSR markers (STU: 174, 182, 190, 200 bp; and STIKA: 195, 198, 219, 221, 223, 242, 245, 248 bp) were revealed for genetic fidelity purpose. A few DUS descriptors of MSH/14-7 are: white-green sprout, compact plant foliage, tall plant height, green stem colour, intermediate leaf structure, large leaf length, broad leaf width, no anthocyanin coulouration on flower bud, white

flower corolla, small flower size, yellow anther colour, irregular anther cone, irregular pistil, equal stylar length, light yellow tuber skin colour, smooth skin, ovoid shape, shallow eye depth and light yellow tuber flesh colour. This interspecific potato hybrid with diverse genetic background, high yield and moderate resistance to late blight disease under hot-spot conditions has potential to employ in potato breeding programmes to widen the genetic base of the cultivated gene pool.

References

Chandel P, Tiwari JK, Ali N, Devi S, Sharma Shashi, Sharma Sanjeev, Luthra SK and Singh BP (2015) Interspecific potato somatic hybrids between *Solanum tuberosum* and *S. cardiophyllum*, potential sources of late blight resistance breeding. *Plant Cell Tissue and Organ Culture* **123:**579–589.

Tiwari JK, Devi S, Ali N, Luthra SK, Kumar V, Bhardwaj V, Singh RK, Rawat S and Chakrabarti SK (2017) Progress in somatic hybridization research in potato during the past 40 years. *Plant Cell Tissue and Organ Culture* **132:** 225-238

Tiwari JK, Luthra SK, Devi S, Kumar V, Ali N, Zinta R and Chakrabarti SK (2018) Development of advanced back-cross progenies of potato somatic hybrids and linked ISSR markers for late blight resistance with diverse genetic base- first ever produced in Indian potato breeding. *Potato J*, **45 (1):** 17-27

59. CPH62 (IC640714; INGR21170), a diploid potato (*Solanum Cardiophyllum* Lindl) germplasm highly resistant to late blight disease and somatic hybridization

Jagesh Kumar Tiwari[1*], Vinod Kumar[1], Sanjeev Sharma[1], Dalamu[1], Rajesh K Singh[1], Tanuja Buckseth[1], Satish K Luthra[2], Vinay Bhardwaj[1] and Manoj Kumar[1]

[1]*ICAR-Central Potato Research Institute, Shimla – 171001, Himachal Pradesh, India*

[2]*ICAR-CPRI Regional Station, Modipuram Campus, Meerut-250110, Uttar Pradesh, India*

**Email: jageshtiwari@gmail.com*

An elite genetic stock CPH62 was developed at ICAR-Central Potato Research Institute, Shimla by Clonal Selection method from several clones regenerated from true potato seed (TPS) of wild species (*Solanum cardiophyllum*, Accession no. PI283062). CPH62 is an important genetic material for fusion parent in somatic hybridization and parental line in diploid breeding programme for introgression of very high resistance to late blight trait with wider genetic base into cultivated potato and also basic research studies on diploid potato breeding. Since potato is native to Lima, Peru (South America) and under the routine germplasm procurement programme of the institute. Our finding shows successful utilization of clonal selection method in development of elite genetic stock CPH62 for high resistance to late blight over four seasons under controlled conditions by challenge inoculation. DNA fingerprinting of CPH62 revealed SSR alleles (STU: 175, 178, 182, 187, 197, 202, 206, 209 bp, and STIIKA: 195, 203, 209, 214, 223 bp) for genetic fidelity. A few DUS descriptors of CPH62 are: purple lightsprout, semi-compact plant foliage, tall plant height, green stem colour, intermediate leaf structure, small leaf length, narrow leaf width, ovate lanceolate leaflet shape, blue-violet flower corolla, small flower corolla size, yellow anther colour, normal anther cone, normal pistil, longer stylar length, late maturity, white cream tuber skin colour, smooth skin type, ovoid tuber shape, shallow eye depth and white tuber flesh colour. This diploid wild species CPH62 with diverse genetic background having very high resistance to late blight disease has potential to widen the gene pool of the cultivated potato.

References

Chandel P, Tiwari JK, Ali N, Devi S, Sharma SH, Sharma SA, Luthra SK and Singh BP (2015). Interspecific potato somatic hybrids between *Solanum tuberosum* and *S. cardiophyllum*, potential sources of late blight resistance breeding. *Plant Cell Tissue and Organ Culture* **123:**579–589.

Tiwari JK, Ali S, Devi S, Zinta R, Kumar V and Chakrabarti SK (2019). Analysis of allelic variation in wild potato (Solanum) species by simple sequence repeat (SSR) markers. *3Biotech,* 9: 262

Tiwari JK, Devi S, Ali N, Luthra SK, Kumar V, Bhardwaj V, Singh RK, Rawat S and Chakrabarti SK (2017). Progress in somatic hybridization research in potato during the past 40 years. *Plant Cell Tissue and Organ Culture,* **132:** 225-238

Tiwari JK, Devi S, Sharma SA, Chandel P, Rawat S and Singh BP (2015) Allele mining in *Solanum* germplasm: cloning and characterization of RB-homologous gene fragments from late blight resistant wild potato species. *Plant Molecular Biology Reporter,* 3**3:**1584–1598.

60. PNT43 (IC640715; INGR21171), a diploid potato (*Solanum pinnatisectum* Dunal) germplasm highly resistant to late blight disease and suitable for protoplast fusion and somatic hybrid development.

Jagesh Kumar Tiwari[1*], Vinod Kumar[1], Sanjeev Sharma[1], Dalamu[1], Rajesh K Singh[1], Tanuja Buckseth[1], Satish K Luthra[2], Vinay Bhardwaj[1] and Manoj Kumar[1]

[1]*ICAR-Central Potato Research Institute, Shimla –171001, Himachal Pradesh, India*

[2]*ICAR-CPRI Regional Station, Modipuram Campus, Meerut-250110, Uttar Pradesh, India*

**Email: jageshtiwari@gmail.com*

An elite genetic stock PNT43 was developed at ICAR-Central Potato Research Institute, Shimla by Clonal Selection method from several clones regenerated from true potato seed (TPS) of wild species (*Solanum pinnatisectum*, Accession no. CGN17443). PNT43 is an important genetic material for fusion parent in somatic hybridization and parental line in diploid breeding programme for introgression of very high resistance to late blight trait with wider genetic base into cultivated potato and also basic research studies on diploid potato breeding. Since potato is native to Lima, Peru (South America) and under the routine germplasm procurement programme of the institute. Our finding shows successful utilization of clonal selection method in development of elite genetic stock PNT43 for high resistance to late blight over four seasons under controlled conditions by challenge inoculation. DNA fingerprinting of PNT43 revealed SSR alleles (STU: 171, 175, 178, 187, 190, 202 bp, and STIIKA: 192, 195, 203, 209, 214, 218, 223 bp) for genetic fidelity. A few DUS descriptors of PNT43 are: purple lightsprout, open plant foliage, small plant height, green stem colour, open leaf structure, small leaf length, narrow leaf width, narrow lanceolate leaflet shape, white flower corolla, small flower corolla size, yellow anther colour, normal anther cone, normal pistil, longer stylar length, late maturity, white tuber skin colour, rough skin type, round tuber shape, shallow eye depth and white tuber flesh colour. This diploid wild species PNT43 with diverse genetic background having very high resistance to late blight disease has potential to widen the gene pool of the cultivated potato.

References

Sarkar D, Tiwari JK, Sharma SU, Poonam, Sharma SA, Gopal J, Singh BP, Luthra SK, Pandey SK and Pattanayak D (2011). Production and characterization of somatic hybrids between *Solanum tuberosum* L. and *S. pinnatisectum* Dun. *Plant Cell Tissue and Organ Culture,* **107:** 427-440

Tiwari JK, Ali S, Devi S, Zinta R, Kumar V and Chakrabarti SK (2019). Analysis of allelic variation in wild potato (Solanum) species by simple sequence repeat (SSR) markers. *3Biotech* 9: 262

Tiwari JK, Devi S, Ali N, Luthra SK, Kumar V, Bhardwaj V, Singh RK, Rawat S, Chakrabarti SK (2017) Progress in somatic hybridization research in potato during the past 40 years. *Plant Cell Tissue and Organ Culture,* **132:** 225-238

Tiwari JK, Devi S, Sharma SA, Chandel P, Rawat S and Singh BP (2015). Allele mining in *Solanum* germplasm: cloning and characterization of RB-homologous gene fragments from late blight resistant wild potato species. *Plant Molecular Biology Reporter,* **33:**1584–1598.

61. STO61 (IC640716; INGR21172), a diploid potato (*Solanum stoloniferum* Schltdl. & Bouché) germplasm highly resistant to late blight disease

Jagesh Kumar Tiwari[1*], Vinod Kumar[1], Sanjeev Sharma[1], Dalamu[1], Rajesh K Singh[1], Tanuja Buckseth[1], Satish K Luthra[2], Vinay Bhardwaj[1] and Manoj Kumar[1]

[1]*ICAR-Central Potato Research Institute, Shimla – 171001, Himachal Pradesh, India*

[2]*ICAR-CPRI Regional Station, Modipuram Campus, Meerut-250110, Uttar Pradesh, India*

[*]*Email: jageshtiwari@gmail.com*

The elite genetic stock STO61 was developed at ICAR-Central Potato Research Institute, Shimla by Clonal Selection method from several clones regenerated from true potato seed (TPS) of wild species (*Solanum stoloniferum*. Accession no. PI225661). STO61 is an important genetic material for fusion parent in somatic hybridization and parental line in diploid breeding programme for introgression of very high resistance to late blight trait with wider genetic base into cultivated potato and also basic research studies on diploid potato breeding. Since potato is native to Lima, Peru (South America) and under the routine germplasm procurement programme of the institute. Our finding shows successful utilization of clonal selection method in development of elite genetic stock STO61 for high resistance to late blight over four seasons under controlled conditions by challenge inoculation. DNA fingerprinting of STO61 revealed SSR alleles (STU: 171, 178, 184, 190, 193, 197, 202 bp, and STIIKA: 188, 195, 209, 214, 230, 234 bp) for genetic fidelity. A few DUS descriptors of STO61 are: purple lightsprout, semi-compact plant foliage, small plant height, dark purple stem colour,

intermediate leaf structure, small leaf length, narrow leaf width, ovate lanceolate leaflet shape, white flower corolla, small flower corolla size, yellow anther colour, normal anther cone, normal pistil, longer stylar length, late maturity, brown tuber skin colour, rough skin type, round tuber shape, shallow eye depth and cream tuber flesh colour. This diploid wild species STO61 with diverse genetic background having very high resistance to late blight disease has potential to widen the gene pool of the cultivated potato.

References

Tiwari JK, Ali S, Devi S, Zinta R, Kumar V and Chakrabarti SK (2019). Analysis of allelic variation in wild potato (Solanum) species by simple sequence repeat (SSR) markers. *3Biotech,* 9: 262

Tiwari JK, Devi S, Ali N, Luthra SK, Kumar V, Bhardwaj V, Singh RK, Rawat S and Chakrabarti SK (2017). Progress in somatic hybridization research in potato during the past 40 years. *Plant Cell Tissue and Organ Culture,* **132:** 225-238

Tiwari JK, Devi S, Sharma SA, Chandel P, Rawat S and Singh BP (2015). Allele mining in *Solanum* germplasm: cloning and characterization of RB-homologous gene fragments from late blight resistant wild potato species. *Plant Molecular Biology Reporter,* **33:**1584–1598.

Tiwari JK, Kumar V, Zinta R, Dalamu, Bhardwaj V, Sharma S, Kumar M and Chakrabarti SK (2019). Characterization of wild potato species for molecular, morphological and late blight resistance traits. *Potato Journal,* **46:** 107-114.

62. MSH/17-16 (IC640717; INGR21173), interspecific somatic hybrid-derived potato (*Solanum tuberosum* L.) germplasm with wider genetic base, yellow tuber flesh colour and high carotenoids content

Jagesh Kumar Tiwari[1*], Satish K Luthra[2], Dalamu[1], Pinky Raigond[1], Mehi Lal[2], Rajesh K Singh[1], Brajesh Singh[1], Tanuja Buckseth[1], Vinod Kumar[1], Vinay Bhardwaj[1], Ashwini K Sharma[1] and Manoj Kumar[1]

[1]*ICAR-Central Potato Research Institute, Shimla-171001, Himachal Pradesh, India*

[2]*ICAR-CPRI Regional Station, Modipuram Campus, Meerut-250110, Uttar Pradesh, India*

[*]*Email: jageshtiwari@gmail.com*

Interspecific potato hybrid MSH/17-16 is a back-cross progeny of advanced stage clonal selection at fourth generation (BC_1-C_4). MSH/17-16 was developed by crossing between Kufri Garima and interspecific potato somatic hybrid clone 'Crd10'. The clone 'Crd10' is an interspecific somatic hybrid, which was developed by protoplast fusion between *Solanum tuberosum* dihaploid 'C-13' and diploid wild potato species *S. cardiophyllum*. This MSH/17- 16 hybrid possesses wider genetic base into cultivated (*S. tuberosum*) from the diploid wild potato species (*S. cardiophyllum*) and possesses yellow tuber flesh colour with high carotenoids content, and also produces medium range of tuber yield (32.78 t/ha) under sub- tropical plain conditions at Modipuram, Meerut (UP). This shows successful utilization of somatic hybrid 'Crd10' in developing a promising advanced stage hybrid (MSH/17-16) with yellow tuber flesh colour and high carotenoids content for use as a parental line in potato breeding in future. Additionally, molecular profiling using SSR markers (STU: 174, 182, 190, 200 bp; and STIKA: 195, 198, 221, 223, 231, 242, 245, 248 bp) were revealed for genetic fidelity purpose. A few DUS descriptors of MSH/14-129 are: red-purple sprout, compact plant foliage, small plant height, green predominant stem colour, intermediate leaf structure, small leaf length, narrow leaf width, no *anthocyanin* coulouration on flower bud, white flower corolla, small flower size, yellow anther colour, irregular anther cone, normal pistil, longer stylar length, yellow tuber colour, smooth skin, ovoid tuber shape, shallow eye depth and yellow tuber flesh colour. This interspecific potato hybrid with diverse genetic background having yellow tuber flesh colour and high carotenoids content has potential to employ in potato breeding programmes to widen the genetic base of the cultivated potato.

References

Chandel P, Tiwari JK, Ali N, Devi S, Sharma Shashi, Sharma Sanjeev, Luthra SK and Singh BP (2015). Interspecific potato somatic hybrids between *Solanum tuberosum* and *S. cardiophyllum*, potential sources of late blight resistance breeding. *Plant Cell Tissue and Organ Culture,* **123:** 579–589.

Tiwari JK, Devi S, Ali N, Luthra SK, Kumar V, Bhardwaj V, Singh RK, Rawat S and Chakrabarti SK (2017). Progress in somatic hybridization research in potato during the past 40 years. *Plant Cell Tissue and Organ Culture,* **132:** 225-238

Tiwari JK, Luthra SK, Devi S, Kumar V, Ali N, Zinta R and Chakrabarti SK (2018). Development of advanced back-cross progenies of potato somatic hybrids and linked ISSR markers for late blight resistance with diverse genetic base- first ever produced in Indian potato breeding. *Potato J*, **45 (1):** 17-27

Tiwari *et al* (2021) Identified candidate genes for yellow tuber flesh colour in potato. NCBI submission (BioProject: PRJNA713962; BioSamples: Shoot- SAMN18275726 and SAMN18275727; Stolon- SAMN18275734 and SAMN18275735; SRA submission: Shoot- SRR14134357 and SRR14134356; Stolon- SRR14134362 and SRR14134363)

63. NUE/15-8 (IC640718; INGR21174), a potato (*Solanum tuberosum* L.) germplasm with high yield under low nitrogen (50 kg N/ha) supply in field conditions and suitable for low input agriculture

Jagesh Kumar Tiwari*[1], Devendra Kumar[1], Vijay K Dua[1], Vinod Kumar[1], Rajesh K Singh[1], Pinky Raigond[1], Tanuja Buckseth[1], Brajesh Singh[1], Sanjay Rawal[2], Dalamu[1], Satish K Luthra[1], Vinay Bhardwaj[1] and Manoj Kumar[1]

[1]*ICAR-Central Potato Research Institute, Shimla –171001, Himachal Pradesh, India*

[2]*ICAR-CPRI Regional Station, Meerut-250110, Uttar Pradesh, India*

**Email: jageshtiwari@gmail.com*

Global agriculture faces problems of environmental pollution due to excess application of nitrogen (N) fertilizers in potato. Hence, aim of this study was to develop potato hybrids with enhanced nitrogen use efficiency (NUE) and yield traits under limited N availability. We generated here a bi-parental population of 116 progenies by crossing two contrasting varieties *viz*., Kufri Jyoti (N inefficient) and Kufri Gaurav (N efficient). After six years (2015-21) of breeding, clonal selection and field trials, we developed advance hybrids based on 20 traits of agronomic, physio-biochemical and NUE parameters. The potato hybrid NUE/15-8 was developed by crossing between Kufri Jyoti (N inefficient) and Kufri Gaurav (N efficient) potato varieties and currently it is at sixth clonal generation (F_1C_6). This advance hybrid NUE/15-8 is a nitrogen use efficient and produces about 20% higher yield (47.20 t/ha) than the best control Kufri Gaurav (39.11 t/ha) under limited nitrogen (50 kg N/ha) fertilizer application in the field at ICAR-CPRI, Regional Station, Modipuram (UP). The hybrid NUE/15-8 has significantly ($p < 0.05$) higher Nitrogen Use Efficiency (NUE) parameters than the best control such as NUE (4.11 vs. K. Gaurav: 2.23), Agronomic NUE (AgNUE) (11.79 vs. K. Gaurav: 9.17), Nitrogen Uptake Efficiency (NUpE) (0.12 vs. K. Gaurav: 0.07), and Nitrogen Utilization Efficiency (NUtE) (35.11 vs. K. Gaurav: 34.20). DNA fingerprinting was performed using SSR markers for true-to-type identification (STU: 173, 178, 181, 189, 198, 199 bp; and STIKA: 175, 194, 214, 223, 230, 234, 243, 247 bp). A fewDUS descriptors of NUE/15-8 are: red-purple sprout, compact plant foliage, medium plant height, green predominant stem colour, open leaf structure, medium leaf length, medium leaf width, no anthocyanin coulouration on flower bud, white flower corolla, small flower size, orange anther colour, normal anther cone, normal pistil, longer stylar length, white cream tuber colour, smooth skin, ovoid tuber shape, shallow eye depth and cream tuber flesh colour. This hybrid has potential for cultivation for low input agriculture mainly under limited N.

References

Tiwari JK, Buckseth T, Devi S, Varshney S, Sahu S, Patil VU, Zinta R, Ali N, Moudgil V, Singh RK, Rawat S, Dua VK, Kumar D, Kumar M, Chakrabarti SK, Rao AR and Rai A (2020). Physiological and genome-wide RNA-sequencing analyses identify candidate genes in a nitrogen-use efficient potato cv. Kufri Gaurav. *Plant Physiol. Biochem,* **154:** 171-183.

Tiwari JK, Buckseth T, Zinta R, Saraswati A, Singh RK, Rawat S, Dua VK, Chakrabarti SK (2020). Transcriptome analysis of potato shoots, roots and stolons under nitrogen stress. *Sci. Rep,* **10:**11-52

Tiwari JK, Plett D, Garnett T, Chakrabarti SK and Singh RK (2018). Integrated genomics, physiology and breeding approaches for improving nitrogen use efficiency in potato: translating knowledge from other crops. *Funct. Plant Biol,* **45:** 587–605.

Trehan SP and Singh BP (2013). Nutrient efficiency of different crop species and potato varieties- in retrospect and prospect. *Potato J,* **40:** 1–21.

64. RGM49 (IC628063; INGR21175), a sunflower (*Helianthus annuus* L.) germplasm with resistance to powdery mildew

Vikas V Kulkarni[1*], IS Hankergoud[1] and Govindappa MR[2]

[1]*AICRP on Sunflower, MARS, University of Agricultural Sciences, Raichur-504184, Karnataka, India*

[2]*Krishi Vigyan Kendra (KVK), Hagari-583111, Karnataka*

[*]*Email: vik_gene@rediffmail.com*

Sunflower (*Helianthus annuus* L.) is one of the important edible oilseed crops grown in the world after soybean and groundnut. It is an important source of edible and nutritious oil. The full potential of this crop is far from being exploited due to several abiotic and biotic stresses. The crop suffers from many fungal diseases, among them foliar disease takes a heavy toll by reducing the yield to considerable extent. Among the foliar diseases, powdery mildew caused by the obligate parasite *Golovinomyces cichoracearum* DC (formerly known as *Erysiphe cichoracearum*) is a potential destructive disease in recent years causing severe yield loss. Since decade, disease observed regularly during *rabi*-

summer seasons and under severe conditions disease is found infecting the cotyledonary leaves up to ray florets. Application of fungicides to manage the disease involves high cost, besides the environmental concern and the insensitivity built up in the pathogen limit their usage (Gullino and Kuijpers, 1994). The higher severity has been observed in tropical parts of the world, where it advances senescence of plant at the flowering or post flowering stages (Gulya *et al.,* 1997).

Breeding efforts were initiated to develop powdery mildew resistant sunflower lines at Main Agricultural Research Station, Raichur during 2011-12. It was aimed to incorporate powdery mildew resistance in some of the elite lines of sunflower through intraspecific and interspecific crosses followed by selections to identify powdery mildew resistant lines (Vikas Kulkarni *et al*., 2015). Raichur Germplasm (RGM-49) was developed from an intraspecific cross between *GM-49* multi-headed restorer lines having good combining ability for seed yield and a multi-headed powdery mildew resistant line *RCR1947/3-2*. The RGM-49 has been found to be good combiner for yield and yield contributing characters with highest levels mid-parent heterosis (80 to 216%) for seed yield (Rudragouda *et al*., 2017). It has black colour seeds with oil content ranging from 35 to 37 per cent. Several earlier reports are available for powdery mildew resistant line in wild relatives and pre-breed inter-specific crosses of sunflower (Reddy *et al*., 2013). However, RGM-49 is the first multi-headed intra-specific cross derivative with cultivated background which can be directly used as restorer line for hybrid development.

References

Gullino ML and LAM Kuijpers (1994). Social and political implications of managing plant diseases with restricted fungicides in Europe. *Annual Review of Phytopathology,* **32:** 559–579.

Gulya, TJ Rashid, KY and Masirevic SM (1997). sunflower diseases, sunflower technology and production. *Agron. Monogr, ASA, CSSA, and SSSA, Madison. WI*, pp. 21–65.

Reddy KP, Rao SC, Kirti BP and Sujatha M (2013). Development of a scoring scale for powdery mildew (*Golovanomyces cichoracearum* (DC.) V.P. Heluta) disease and identification of resistance sources in cultivated and wild sunflowers. *Euphytica,* **190:** 385–399.

Rudra Gouda Mali Patil, Vikas V. Kulkarni, Mallikarjun Kenganal, I. Shankergoud and JR Diwan, (2017). Combing ability studies in restorer lines of sunflower (*Helinathus annuus* L.). *J. Appl. Nat. Sci.*, **9(1):** 603-608.

Vikas V. Kulkarni, I. Shankergoud and M.R. Govindappa, 2015, Identification of sunflower powdery mildew resistant sources under artificial screening, SABRAO Journal of Breeding and Genetics, 47 **(4):** 502-509.

GUIDELINES TO AUTHORS

GENERAL

The Indian Journal of Plant Genetic Resources (IJPGR) is the official publication of the Indian Society of Plant Genetic Resources. For publication in the journal, the authors must be a member of the Society. IJPGR publishes full-length papers or short communications of original scientific research in the field of plant genetic resources. Review articles (with prior consent or invitation only) summarizing the existing state of knowledge in topics related to plant genetic resources will also be published.

Contributions should be as concise as possible. The maximum length of the review article, full-length papers and short communications is usually restricted to 12, 6, 3 printed pages including illustrations and tables, respectively.

SCOPE

- lBasic research on biosystematics, genetics and genomics related to PGR
- lApplied research on field and laboratory evaluation of PGR
- lSupportive research in conservation and quarantine of PGR
- lPolicy research on access and benefit sharing; IPRs
- lStatus reports on ecology, conservation, traditional knowledge and use of PGR

EDITORIAL BOARD

N. SIVARAJ
Principal Scientist
ICAR-NBPGR Regional Station – Hyderabad
ARI Campus, Rajendra Nagar
Hyderabad-500 030, Andhra Pradesh
n.sivaraj@icar.gov.in

R.K. SALGOTRA
Professor & Coordinator
School of Biotechnology
Sher-e-Kashmir University of Agricultural Sciences &
Technology of Jammu-180 009
rks_2959@rediff mail.com

Rakesh SINGH
Principal Scientist
Division of Genomic Resources
National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi-110 012
rakesh.singh2@icar.gov.in

Lalit ARYA
Principal Scientist
Division of Genomic Resources
ICAR-NationalBureau of PlantGenetic Resources
Pusa Campus, New Delhi-110 012
lalit.arya@icar.gov.in

K. PRADHEEP
Principal Scientist
ICAR-NBPGR Regional Station - Thrissur, Vellanikkara,
KAU P.O. Thrissur-680656, Kerala
k.pradheep@icar.gov.in

Sherry Rachel JACOB
Senior Scientist
Division of GermplasmConservation
ICAR-NationalBureau of PlantGenetic Resources
Pusa Campus, New Delhi-110 012
sherry.jacob@icar.gov.in

S. RAJKUMAR
Senior Scientist
Division of Genomic Resources
ICAR-National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi-110 012
S.Rajkumar@icar.gov.in

Kuldeep TRIPATHI
Scientist
Division of Germplasm Evaluation
ICAR-National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi-110 012
kuldeep.tripathi@icar.gov.in

Vandana TYAGI
Principal Scientist
Germplasm ExchangeUnit
ICAR-NationalBureau ofPlantGenetic Resources
Pusa Campus, New Delhi-110 012
vandana.tyagi@icar.gov.in

Kavita GUPTA
Principal Scientist
Division of Plant Quarantine
National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi-110 012
kavita.Gupta@icar.gov.in

Sheikh M. SULTAN
Principal Scientist & Offi cer-In-Charge
ICAR-NBPGR, Regional Station
Srinagar-190 005
Jammu & Kashmir
mohmmad.sheikh@icar.gov.in

Manjusha VERMA
Principal Scientist
Division of Genomic Resources
ICAR-NationalBureau of Plant Genetic Resources
Pusa Campus, New Delhi-110 012
manjusha.verma@icar.gov.in

Amit SINGH
Senior Scientist
Division of Genomic Resources
ICAR-National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi-110 012
Amit.singh5@icar.gov.in

C.S. MOHANTY
Principal Scientist
CSIR-National Botanical Research Institute
436, Rana Pratap Marg, Prem Nagar, Hazratganj
Lucknow-226 001, Uttar Pradesh
cs.mohanti@nbri.res.in

Rakesh BHARDWAJ
Principal Scientist
Division of Germplasm Evaluation
ICAR-National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi-110 012
Rakesh.Bhardwaj1@icar.gov.in

Rajiv KUMAR
Principal Scientist (Horticulture)
Division of Flower and Medicinal Crop
ICAR-Indian Institute of Horticultural Research
Hessaraghatta lake post, Bengaluru-560089, Karnataka
rajiv.kumar11@icar.gov.in

Somnath ROY
Scientist
Central Rainfed Upland Rice Research Station
ICAR-National Rice Research Institute
Hazaribag-825 301, Jharkhand
somnath.roy@icar.gov.in

ORGANIZATION OF THE MANUSCRIPT

Full-length papers

Title Page: The title page of the manuscript should be the first page and should include the title, names and addresses of the authors, abstract and keywords.

Title: Keep the title brief, specific and informative and amendable to indexing. It should be typed in running text with first letter of word as capital and latin names in italics.

Name and Address: The name of authors and the address of the institution where the work was carried out should be mentioned below the title. Present address of correspondence, if different, should be given as footnote indicating by asterisk (*) the author to whom the correspondence and reprint requests are to be made. E-mail addresses should also be indicated, if any.

Abstract: The abstract should clearly state the rationale, objectives, methods, and important conclusions of the study. It should not exceed 150 words.

Key Words: The abstract should be followed by not more than five key words indicating the contents of the paper and useful for abstracting purposes.

Main Text: The main text of the paper should start from the second page which should contain the title of the paper followed by the text divided into following main headings which are to be typed in running text and flushed with the margin: Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References. Wherever appropriate, results and discussion can be combined and acknowledgements be omitted.

Introduction should be brief and limited to the statement of the problem and aim of the experiment.

Materials and Methods should include relevant details on the nature of material, experimental design, the techniques employed and the statistical method used. For well-known methods, citation of reference will suffice.

Results and Discussion should be clear to readers in different disciplines. Units of measurement should be SI.

Tables should be typed on separate sheets, each with a heading stating its contents clearly and concisely. Numerical data and calculations should be thoroughly checked.
Figures of only good quality that are essential to a clear understanding of the paper shall be accepted. Legends to the illustrations should be typed on separate paper. Information in the legend should not be repeated in the text and similarly, the same data should not be represented in both graph and table form. All figures, whether photographs, maps, graphs or line drawings should be numbered consecutively. Illustration number and title of the article with authors' name should be given at the back of the plates in soft pencil.

Line drawings of high quality, preferable in the desired final size would be accepted. The inscriptions should be clearly legible.

Photographs for publication should be of high contrast, black and white, glossy print, trimmed at right angles. Magnification should be indicated with a bar scale on the photo. Authors need to indicate colour reproduction of photographs (cost of colour printing will be borne by the authors).

Acknowledgements should mention only guidance or assistance received in real terms, and financial grant provided by an agency. Acknowledgements for inspiration, typing etc., need not be mentioned.

References in text should be cited by author, year of publication (e.g. Joshi, 1995) and multiple citations should be in chronological order (Withers and Englemann, 1998; Rao *et al*., 2001). References should be listed in alphabetical order under the first authors' name. The names of journals should be abbreviated according to the latest edition of the World List of Scientific Periodicals (eds P Brown and GB Stratton), Butterworths, London.

The following examples may be used for citations:

- Chao, CCT. and RR Krueger, (2007). The date palm (Phoenix dactylifera); an overview of biology, uses and cultivation. *Hort. Sci*., **42(5):** 1077-82.
- Muralidharan,CM; S.B.S. Tikka, and Piyush, Verma,(2008) Date palm cultivation in Kachchh, Tech. Bulletin- 2, Date palm Research Station, SDAU, Mundra, p.36.
- Pareek, O.P. and N.N. Sodagar, (1986). Date palm grooves in Kachchh. *Indian Hort.*, **31:** 21-27
- Singh, R.S. (2018). Date palm: an ideal fruit for health and wealth. *Indian Hort. special issue*, **63(5):** 17-21

Short Communications
The style and format as mentioned for full-length papers should be followed for Short Communications. However, the abstract should be restricted to not more than 50 words and the remainder text should be continuous (without headings). Illustrative material should be kept at minimum, usually not more than one table or figure and only few references should be included (not more than 10). Authors can also submit meeting reports after consulting EIC.

Submission of the Manuscript Submission of an article will be held to imply that it has not been previously published or submitted for publication elsewhere. A cover letter including a statement to this effect should be submitted with the manuscript. Article are submitted through online submission system (http://www.indianjournals.com/ijor.aspx?target=manuscript_ submission) or by email to:

Editor-in-Chief, Indian Journal of Plant Genetic Resources
E-mail: ispgr2015@gmail.com

- Experts in the subject will review all the submitted manuscripts and the final decision about the acceptance of the manuscript rests with the Editorial Board. If manuscript is accepted for publication, the revised manuscript should be accompanied by electronic copy on CD or through electronic mail.
- lPublication of a paper in the Journal does not imply the responsibility for an agreement with the statements or view written therein, and rests entirely on the authors thereof.
- lThe authors will receive page proofs, which should be corrected and returned without delay. Corrections must be kept to the minimum, and the proof stage should not be regarded as an opportunity for further editing and additions. Although, every effort is made by the editors to correct proofs of all the papers they assume no responsibility for errors that may remain in the final print.

ETHICS STATEMENT

IJPGR approves the spirit of the guidelines for journal editors developed by the Committee on Publication Ethics (COPE).
1. Manuscripts submitted to IJPGR are evaluated entirely on the basis of their scientific content and relevance to PGR research community.
2. IJPGR does not levy any publication charges to members of ISPGR.
3. ISPGR, the publishers of the Journal, take all possible measures to uphold the highest standards of publication ethics and to prevent any malpractices.
4. Authors who submit manuscripts to IJPGR declare that the submissions are original and unpublished and are not under consideration for publication elsewhere.
5. Authors also declare that the manuscript is based on their own original work devoid of any nature and amount of plagiarism.
6. IJPGR makes full efforts to eliminate any kind of conflict of interest of authors, editors or reviewers, resulting from competitive, collaborative or other relationship with any person, institution or agency connected to the submissions.

Ethics expected to be followed by IJPGR Editors

1. IJPGR Editorial Team is fully responsible for the decision on acceptance or rejection of a manuscript submitted to the Journal. An established procedure is followed to ensure unbiased decision making. Submissions are handled by different editors based on their specialization. Editors obtain the reports of three referees with expertise in the topic dealt in the submission. Each editor recommends rejection or acceptance (direct or after revisions) to the Editor-in-Chief who takes the final decision.
2. The evaluation of manuscripts is made on the basis of their scholarly content in terms of relevance, novelty and scope as well as their presentation in terms of language and syntax. IJPGR does not consider any other factors like gender, race, religious belief, ethnic origin, citizenship, or political philosophy of the authors.
3. IJPGR strictly follows transparency in the evaluation process and confidentiality of the submission. Reviewers, as on today, remain anonymous to authors.

Ethics expected to be followed by IJPGR Reviewers

1. Peer review is the backbone of IJPGR manuscript flow. IJPGR depends upon the services of reviewers for maintaining ethical integrity and scholarly quality.
2. IJPGR does not follow double blind review and therefore the reviewers are expected to maintain absolute confidentiality with regard to the contents of manuscripts.
3. The reviews are conducted objectively with every comment/decision supported by clear reasons. Reviewers follow a non- ranking format provided by the IJPGR to facilitate decision making by Editor.

Ethics expected to be followed by Authors

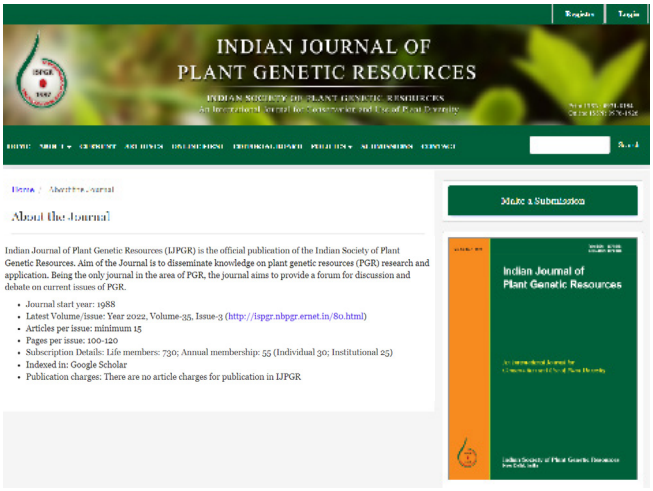
1. Authorship should be restricted to only those persons who have made a significant contribution to the conception, design, execution, or interpretation of the reported study. In recent times, symbiotic authorship has become a serious ethical issue.
2. The authors must ensure that the experiment and manuscript are both entirely original works. Every instance of use of work and/or words of other authors must be appropriately cited or quoted. Any kind of plagiarism detected at review stage results in rejection of the manuscript. If the plagiarism is detected post-publication, the article will be summarily retracted. If any fundamental errors are found in published papers, authors will inform IJPGR which will take corrective measures including retraction.
3. IJPGR persuades authors to provide the basic data related to manuscripts such as description of germplasm and its availability to ensure greater utilization of diverse germplasm. Authors are also encouraged to provide relevant raw data for editorial review. Authors must prepare such data in a format to provide public access. Specific requests of data confidentiality shall be entertained by IJPGR on case to case basis.
4. Authors must avoid any kind of duplication in their submissions i.e. attempting to publish same/significantly similar manuscripts in more than one journal. IJPGR does not consider the following as a prior publication: (i) Abstract in a conference/ symposium; (ii) Academic thesis/dissertation; (iii) Invited talks and interviews on specific topics.
5. Submission to IJPGR implies that authors have complied with ethical and technical standards of use of hazardous material, use of animal or human subjects, access and use of genetic resources, access and use of IP protected data. It is authors' duty to ensure no national or international legal requirement is compromised.
6. Submission of a manuscript to IJPGR implies that the corresponding author has ensured that all co-authors have seen and approved the final version of the paper and have agreed to its submission for publication to IJPGR. All sources of financial support should also be disclosed.

PUBLISHER/EDITORIAL OFFICE ADDRESS

H-204, ICAR-National Bureau of Plant Genetic Resources Pusa Campus, New Delhi–110012, India
E-mail: ispgr2015@gmail.com
Publisher website: www.nbpgr.ernet.in/ispgr
Journal hosting and online submission of manuscript: http://ispgr.in/

Online Access to IJPGR

All the issues of Indian Journal of Plant Genetic Resources from 1988 [Vol 1(1)] till date [Vol 36(1)] are accessible as pdf files of individual papers.



- The access is provided to *life members only*
- Access is through user ID and password
- Login credentials have been emailed to all life members by ispgr.in (if any life member has not received the user ID and password, please contact IJPGR office at ispgr2015@gmail.com)

- Open the home page of IJPGR at https://ispgr.in/index.php/ijpgr/index You may also reach IJPGR home page via Google search of "Indian Journal of Plant Genetic Resources"
- Click on the LOGIN button and enter the user ID and password
- If you are a new member, then click on the REGISTER option, and register as AUTHOR.
- For a new submission, click on MAKE A SUBMISSION, or check our AUTHOR GUIDELINES.
- The login credentials are for the personal use of IJPGR life members. Please do not share with others

Indian Journal of
Plant Genetic Resources

Print ISSN: 0971-8184
Online ISSN: 0976-1926
Vol. 36 No. 3 2023

CONTENTS

RESEARCH ARTICLE

Study on Genetic Variability and Correlation of Yield Traits in the F3 Population Derived from Wild Introgression Lines of Swarna/*Oryza nivara* **341**
Kavitha Beerelli, Divya Balakrishnan, Jyothi Badri, Senguttuvel P, Gireesh C, Ladhalakshmi D, Tuti MD, Padmavathi G, Subba Rao LV and Sarla Neelamraju

Characterization and Evaluation of Faba Bean (*Vicia faba* L.) Germplasm Collected from Kashmir, India **346**
Sheikh M. Sultan, Susheel K. Raina, Suheel Ahmad and Sheeraz S. Bhat

Genetics of Yield and Fruit Quality Traits in Bitter Gourd (*Momordica charantia* L.) **356**
Vinay N. D, Asima Aamin, Tusar K. Behera, Anilabh D. Munshi, Gograj S. Jat, Shyam S. Dey and Ranjith K. Ellur

Agro-morphological Characterization and Digitization of Job's Tears (*Coix lacryma-jobi* L.) Germplasm: A Minor Cereal Crop of Northeastern India **362**
Laxmisha KM, Kailash C. Bhatt, Mamta Arya, Rakesh Bhardwaj, Dinesh P. Semwal and Veena Gupta

Screening of wild and cultivated Vigna accessions for resistance to bruchid (*Callosobruchus maculatus* F) **371**
Ragul Subramaniyan and Manivannan Narayana

Biophysical and Morphological Basis of Resistance against Linseed (*Linum usitatissimum* L.) Bud fly (*Dasyneura lini* Barnes) **382**
Vinay Kumar, Gautham S, Kavita Gupta, V.K. Biradar, Vikender Kaur, Beena Nair and Ajay Kumar

Elucidating Diversity among Mango (*Mangifera indica* L.) Hybrids based on Morphological Characters using DUS Guidelines **387**
Gulshan Kumar, Manish Srivastav, Sreekanth H.S., Jai Prakash, Sanjay K. Singh and Vinod

Development of Cryoconservation Protocol for In-vitro Shoot Bases of *Allium ampeloprasum* L. for Long-term Conservation **396**
Ravi Gowthami, Subhash Chander, Ruchira Pandey, Muthusamy Shankar and Anuradha Agrawal

Phenotypic Characterization of Crop Genetic Resources of AA-genome Species in Rice (*Oryza spp.*) **402**
Aswin Mahendran, Mahesh C. Yadav, Gopala Krishnan S, Shailesh Tiwari and Tapan K. Mondal

***Circa situm* Conservation of Tree Genetic Resources: A Case Study from the Central Western Ghats of Karnataka, India** **415**
Gudihalli M. Puneeth, Ravi Gowthami, Ramesh Vasudeva, Kerekoppa M. Laxmisha and Sunil Archak

A Note on Taxonomy and Genetic Resource Potential of *Oryza meyeriana* var. *indandamanica*, a Rare Wild Relative of Rice from the Andaman Islands **422**
K Pradheep, R Parimalan, K Joseph John, VA Muhammed Nissar, I Jaisankar, PP Thirumalaisamy and M Latha

Effects of Different Treatments on Seed Germination and Breaking Seed Dormancy in Wild *Luffa* Species **429**
Arvind Nagar, Amish K. Sureja, Chithra Pandey, Manjusha Verma, Lalit Arya3, Nakul Gupta and A.D. Munshi

Effect of Nitrogen Nutrition Stress on Grain Weight, Starch Quality in Bread Wheat **434**
Alka Bharati, Sachin Phogat, Jeet Roy, Amarjeet Kumar, Jayanthi Madhavan and Pranab K. Mandal

Genetic Diversity Analysis of Bitter Gourd (*Momordica charantia* L.) Germplasm using Molecular Markers **442**
Anupam Adarsh, Pankaj Kumar, Bishun D. Prasad, Randhir Kumar and Hemant K. Singh

Comparative Analysis of Nutritional Values, Bioactive Compounds and Antinutrients in Tartary and Common Buckwheat Leaves **449**
Nidhi Joshi, Kumkum Giri, Aditi Biswas, Pawanesh Tamta and Kuldip C. Verma

Plant Germplasm Registration Notice **455**
Anju Mahendru Singh and Anjali Kak

Guidelines to Authors

Published by the *General Secretary*, Indian Society of Plant Genetics Resources, NBPGR Campus, New Delhi-110012 and
Printed by *MRI Publication Pvt Ltd.*, Lucknow, Uttar Pradesh. Email ID: info@mripub.com