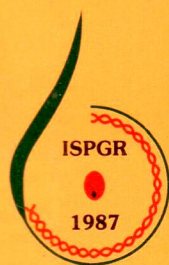


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An Analysis of Parental Dose for Future Sugarcane Varieties

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Development of sub-tropical varieties of sugarcane with characteristic features like high sugar, thick cane etc from the narrow genetic base of the breeding population in sugarcane prompted the parental study of common varieties. The study indicated the role of parental dose in imparting some of the characteristic features to the common varieties. Also, it suggested a time saving methodology to select and utilize parents for the future breeding programmes.

Key Words: Parental dose, Sugarcane, *Saccharum*

Introduction

The development of first commercial hybrid of sugarcane 'Co 205' in 1918, marked the success of interspecific hybridization programme (Hogarth *et al.*, 1997; Janakiammal, 1941) taken-up by Barber in 1912. The process of development of new varieties derived from such inter-specific and some times tri-specific hybridization schedule gained momentum with this success. With the development of high yielding, high sugar and adapted varieties for different agro-climatic zones, sugarcane breeders started using these varieties as parents in location specific breeding programmes. On one hand, these parents gave some highly adapted varieties for specific locations but along with this, they also caused narrowing of the genetic base of the developed varieties (Nair *et al.*, 1998; Hemprabha and Rangasamy, 2001). The good yield, adaptability and high sucrose content of some the newly developed varieties with the narrow genetic base of the parents deserves detailed analysis (Kennedy, 2001; Breaux, 1984; Heinz, 1987).

For the purpose of this study, twelve leading varieties (Table 1) of sub-tropical India were selected for parental

details. Care was taken that no such variety was selected whose parentage has any unknown parent (like GCs, PCs, etc.). These varieties are well adapted for sub-tropical conditions and they represent most of the diversity in the form of high sugar (CoJ 64, CoS 96268, CoS 8436), high fibre (BO 91, CoSe 92423), early maturity (CoJ 64, CoS 8436, CoS 687), mid-late maturity (CoLk 8001, CoSe 92423, BO 91), thick canes (CoS 8436, CoLk 8001) and so on. In the parental study up to the basic germplasm level, it was found that all these 12 varieties were developed from 18 basic genotypes (Table 2). It is very pertinent to indicate here that out of these 18 genotypes, nine are found in the parentage of all the 12 varieties. The table also indicates the dose of basic parents in each variety. Here, dose refers to the number of times a basic parent is present in the parentage.

The importance of the dose can be viewed upon from different angles. The foremost being the characteristic feature of a variety, which is a direct representative of a specific parental dose (Natarajan *et al.*, 1967; Parthasarathy 1948). The best example, which can be cited from Table 1, is of varieties CoS 8436 and CoLk 8001, which have

Table 1. General characteristics of some common sub-tropical sugarcane varieties

S. No.	Variety	Parentage	General Characteristics
1.	BO 91	BO 55 x BO 43	Mid late maturing, medium thick, solid and hard cane
2.	CoS 687	Co 976 x Co 312	Early maturing, medium thin, solid and soft cane
3.	CoS 767	Co 419 x Co 313	Mid late maturing, medium thick, medium hard and solid cane
4.	Co 1158	Co 421 x Co 419	Mid late maturing, medium thick, medium soft and solid cane
5.	CoLk 8001	Co 62174 x Co 1148	Mid late maturing, thick, medium soft and solid cane
6.	Co 1148	P 4383 x Co 301	Mid late maturing, medium thick, soft and solid cane
7.	CoJ 64	Co 976 x Co 617	Early maturing, high sugar, medium thick, soft and solid cane
8.	CoPant 84212	Co 1148 x Co 775	Mid late maturing, medium thick, soft and solid cane
9.	CoS 96268	Co 1158 x Co 62198	Early maturing, high sugar, medium thick and medium hard cane
10.	CoSe 92423	BO 91 x Co 453	Mid late maturing, medium thick, medium soft and solid cane
11.	CoS 8436	MS 68/47 x Co 1148	Early maturing, high sugar, thick and hard cane with fine pith
12.	CoS 88230	Co 1148 x Co 775	Early maturing, medium thick, soft and solid cane

Table 2. Parental dose of basic germplasm in the pedigree of some sub-tropical sugarcane varieties

S. Basic parents in pedigree No.	Dose of basic parents in pedigree											
	BO 91	CoS 687	CoS 767	Co 1158	CoLk 8001	Co 1148	CoJ 64	CoPant 84212	CoS 96268	CoSe 92423	CoS 8436	CoS 88230
1. Black Cheribon	4	5	4	3	12	5	5	8	8	5	13	8
2. Chunnee	3	4	3	1	7	5	2	6	3	3	7	6
3. Kaludai Boothan	1	2	2	1	3	2	1	2	1	1	2	2
4. <i>S. spontaneum</i> (CBE)	5	6	3	2	4	2	5	2	3	6	3	2
5. Vellai	1	2	1	–	–	–	1	–	–	1	–	–
6. <i>S. spontaneum</i> (Java)	1	1	1	2	6	1	2	3	5	1	7	3
7. Banjer massin Hitam	1	2	2	4	10	2	3	4	6	1	12	4
8. Loethers	1	2	2	4	10	2	3	4	6	1	12	4
9. Lahiana	1	1	1	2	3	1	2	2	4	1	6	2
10. Fidji	1	1	1	2	3	1	2	2	4	1	6	2
11. Ashy Mauritius	1	1	–	–	–	–	1	–	1	1	–	–
12. Green Sport	2	1	–	1	2	–	2	–	1	3	1	–
13. Zwart Cheribon	–	1	–	–	1	–	–	–	–	–	–	–
14. A-2	–	1	–	–	–	–	1	–	–	–	–	–
15. Striped Mauritius	–	–	–	–	1	–	–	1	1	–	1	1
16. White Transparent	–	–	–	1	1	–	–	–	1	–	–	–

Black Cheribon in 13 and 12 doses respectively, imparting their characteristic thick and juicy canes. Also, these two varieties have one more parent Q 116 in common, although it is in single dose. To further unravel the characteristics with reference to parental dose, an attempt was made to detail the first, second and third generation hybrids (Table 2), which are represented in the parentage of the twelve varieties under study.

A close perusal of Tables 1 and 2 confirms the viewpoint that adaptability and resistance had been introgressed from *S. spontaneum* (Coimbatore and Java forms) and *Erianthus* while sugar had been transferred from *S. officinarum* and adaptability to sub-tropical climate of India came from *S. barberi*. Case study of BO 91 would further strengthen the parental dose concept in imparting the characteristic features to a particular variety. BO 91, a popular variety of north-central India, where there is wide spread abiotic stress condition, has *S. barberi* genotype 'Chunnee' in three doses along with *S. officinarum* genotypes 'Black Cheribon', Kaludai Boothan, Vellai, Banjer massin Hitam, Loethers, Lohiana, Fidji, Ashy Mauritius and Green Sport in 4,1,1,1,1,1,1 and 2 doses respectively. Five doses of *S. spontaneum* (Coimbatore form 2n=64) further reinforced it with resistance to major pathotypes of red rot and adaptability to abiotic stresses prevalent in the area.

Another leading variety CoJ 64 has only two doses of *S. barberi* (Chunnee) making it suitable for only a limited zone of north-western India, but the reinforcement from 7 doses of *S. spontaneum* (Coimbatore and Java forms) provided the much needed field resistance to it. The earliness and high sucrose content of this miracle variety can be attributed to the rare combination of *S. officinarum* genes from Black Cheribon (5 doses) with Vellai (1 dose) and A-2 (1 dose). Varieties CoLk 8001 and CoS 8436, which are well known to be suitable for better management conditions, have very high doses of Black Cheribon, Banjer massin Hitam and Loethers along with 7 doses of Chunnee and 10 doses of *S. spontaneum*. This kind of favourable combination of genes for sugar, resistance and adaptability made them varieties of choice for sugar factories and farmers both.

Generation wise analysis of the parents clearly indicated that Black Cheribon was the most important *S. officinarum* parent in the first generation hybrids under nobilisation process. The frequency of its presence in parentage of onward generations had been a major factor for accumulation of sugar genes. Banjer massin Hitam and Loethers were next, providing some rare combinations in first generation (POJ 100) and second-generation hybrid parents (EK-28). Another important basic parent 'Vellai' marked the development of first generation commercial

hybrids, which were later on extensively used as parents indicating that the use of *S. spontaneum* (Coimbatore form 2n=64) is a successful option for transferring red rot resistance and widespread adaptability in the nobilisation process. Variety/ parent Co 205 was unique in the sense that it was the real F₁ commercial hybrid involving only two species of *Saccharum* viz. *S. officinarum* and *S. spontaneum*, confirming their ability to produce commercial gene combinations for sub-tropical conditions, although, both may be of tropical origin.

Other than Co 205, no successful commercial hybrid released for sub-tropical India had been a mix of blood from just two species. Rather, they have a combination of genes from more than two of the major *Saccharum* species viz. *officinarum*, *spontaneum*, *sinense*, *barberi* and *robustum*. Also, they have a combination of *S. spontaneum* blood from Coimbatore form (2n = 64) and Java form (2n = 112), Moreover, with few exceptions, the present day hybrid varieties in cultivation under sub-tropical India, stem from the Javan-Indian hybridization products. This

Table 3. 1st, 2nd and 3rd generation hybrids and their parentage

Hybrid Parents	Parentage
1st Generation Hybrids	
POJ 213	Black Cheribon x Chune
Co 291	Kaludai Boothan x <i>S. spontaneum</i> Coimbatore
POJ 1410	Black Cheribon x Chune
Kassoer	Black Cheribon x <i>S. spontaneum</i> Java
POJ 100	Banjer massin Hitam x Loethers
EK 2	Lahiana x Fidji
POJ 181	Black Cheribon x Chune
Co 285	Green Sport x <i>S. spontaneum</i> Coimbatore
M 2	Kaludai Boothan x <i>S. spontaneum</i> Coimbatore
Co 205	Vellai x <i>S. spontaneum</i> Coimbatore
Co 206	Asy Mauritius x <i>S. spontaneum</i> Coimbatore
2nd Generation Hybrids	
Co 213	POJ 213 x Kansar
Co 214	Striped Mauritius x Saretha/ <i>spontaneum</i> Blood Seedling (M 4600)
POJ 2364	Kassoer x POJ 100
EK 28	POJ 100 x EK 2
Co 221	POJ 213 x M 2
Co 243	A 2 x Co 206
Co 244	POJ 213 x Co 205
Co 453	Black Cheribon x Co 285
POJ 385	POJ 100 x Chune
Co 605	Zwart Cheribon x Co 285
3rd Generation Hybrids	
POJ 2878	POJ 2364 x EK 28
Co 299	Co 213 x POJ 1410
Co 312	Co 213 x Co 244
Co 290	Co 221 x D 74
Co 313	Co 213 x Co 244
Co 331	Co 213 x Co 214
POJ 2725	POJ 2364 x EK 28
Co 371	Co 213 x Co 214

is something, which needs attention when a huge amount of genetic diversity available in the germplasm collection is still waiting for utilization.

Further, going in to the details of these widely used parents indicate that 'Kassoer,' which itself is considered to be natural hybrid of the Cheribon form of *S. officinarum* and Glagah the Javan form of *S. spontaneum*, when used in the classical nobilisation of sugarcane, resulted into the development of world famous hybrid POJ 2878, which became a landmark for adaptability and quality. Other important first generation hybrid parents POJ 13, POJ 1410 and POJ 181 were derived from a common cross between Black Cheribon and Chune, while Co 291, Co 285, M 2, Co 205 and Co 206 have same *S. spontaneum* parent. The parents of second generation viz. Co 213, POJ 2364, EK 28, Co 221 and Co 244 have either POJ 213 or POJ 100 as one of the ancestors again emphasizing the repetitive dose of Black Cheribon and Banjer massin Hitam. The value of Chune in imparting adaptability can also be viewed through POJ 213, POJ 1410, POJ 181, etc. which gave rise to many varieties for Hawaiian sugarcane cultivation.

The planning for future sugarcane varieties revolves around two major features viz. their sugar producing ability and high level of field resistance against major pathotypes of red rot pathogen. Based on the analysis of parents of the existing varieties, it can be clearly pointed that so far, whatever gains have been obtained in the sugar recovery front, are only due to the doses of Black Cheribon, Banjer massin Hitam and Loethers in the parentage. This indicates that, for development of high sugar parental genotypes two simultaneous strategies can be followed. The first strategy would be initiating nobilisation process afresh with basic germplasm like Black Cheribon and *S. spontaneum*, which may take years to yield desired results. It is because of the fact that there are huge untapped basic germplasm resources maintained in the World Collection and the possibility of making all the cross combinations among them is remote. Moreover, when new genotypes are brought in the breeding pool, their efficiency in expression of desired characters needs rigorous testing in terms of adaptability and over the year repeatability. This requires long-term research projects targeted to produce good parental lines with high combining abilities. But in a crop like sugarcane, where parental assessments are usually based on performance *per se* rather than biometrically analyzed gene-actions, it would be pertinent to use only those parents which are

expected to give favorable results. Thus, the focus may now be shifted to the second strategy, which involves broadening of genetic base of those varieties, which already have high parental dose of Black Cheribon, Banjer massin Hitam and Loethers. The probability of combining high sugar yields with higher field resistance to red rot pathotypes, will be much more when intra-specifically improved *S. spontaneum* (improved through Indian x Javan crosses) parents will be crossed with such varieties. This will save time required in the rigorous testing schedule and would produce parental lines as well as varieties adapted to the sub-tropical conditions and farmer's need.

Conclusion

The problem of narrow genetic base of sugarcane varieties will continue to hinder the breeding achievements in desired direction but by selecting parents with higher doses of a particular basic germplasm can provide some solution to overcome this situation. The future sugarcane varieties can be developed on the basis of parental doses for desired expression of specific characters. Also, for introgression of new genes from the so far unutilized *Saccharum* gene pool, such studies will be required to identify best backgrounds.

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Identification of Landraces of Rice from Jharkhand and Bihar Resistant to Bacterial Blight

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Bacterial leaf blight of rice, the second most devastating disease of rice causes substantial yield loss. Study of pathogenic variability and identification of resistance genes are key factors in breeding against this disease. Bacterial leaf blight is now a serious constraint for rice production in the irrigated and low land ecologies in all rice growing countries. 223 lines (landraces) were collected from different regions of Bihar and Jharkhand and were screened against bacterial blight. Out of 223 lines, eight lines, namely Bhathani, Hardi Muri, Sitwa dhan, Jhulat, Lambasari, Karijiri, Swarna gora and Sita gora were found to be highly resistant.

Key Words: Basmati rice, Bacterial Blight (BB), Virulence, Land race

Introduction

Rice is a major food crop of the world and in India also 2/3rd of the population uses it in various forms. In India rice is cultivated round the year in one part or the other of country; in diverse ecologies spread over 44 million hectares with a production of 90 million tons. One of the major reasons of low productivity is damage by insects-pests and diseases, which cause an annual loss of 10-15% to rice yield

Bacterial Blight caused by *Xanthomonas oryzae* pv *oryzae* (Ishiyama) Swing *et al.* (1990), Xoo is the second most important disease of rice after blast and most important bacterial disease in terms of economic loss. It causes an annual loss of 20-30% in Japan and 6-60% in India. It caused major epidemic in Punjab, Haryana and western Uttar Pradesh in 1979 and 1980 and caused complete destruction of crop (Durgapal, 1985). It has been observed that none of the designated resistance genes produce resistance to most isolates found in Punjab and Haryana. Indian traditional rice germplasms may contain resistance genes that may be more suitable to counter act virulent Indian patho-types more effectively than the resistance genes identified abroad. Considering this, 223 germplasms were tested against the most virulent and aggressive Xoo isolate for identifying new resistance sources.

Materials and Methods

Two hundred and twenty three traditional rice line (landraces) were collected from Jharkhand and Bihar including some lines received through NGO named Gene Campaign, Ranchi.

These lines were grown at Division of Genetics, IARI, New Delhi in randomized block design in three replications during *Kharif* 2006. All these germplasm lines were grown at CRRI Cuttack in *Rabi* 2007 also. Each line was grown as a single row of 3m length with a distance of 60 X 20 cm between rows and plants, respectively. Recommended agronomical practices were followed to raise the crop.

These germplasms were artificially inoculated with most virulent and aggressive Xoo isolate by leaf tip clipping method (Satya *et al.*, 2004). The concentration of bacterial suspension used for inoculation was 10¹⁰ cells /ml (Mew, 1987). Standard Evaluation System (SES) advocated by IRRI for scoring the resistance based on the percent area infected by the pathogen after a certain interval was used. Decision of degree of resistant or susceptible (0->15.0) is taken after 15 days of inoculation. The observations were taken on the basis of average lesion length (cm) of ten leaves per plant (Table 1) as per scale proposed by Ogawa (1993).

Results and Discussion

The results of data are presented in Table 1. It can be observed that out of 223 germplasms only 13 lines as resistant, 168 lines as moderately resistant, 27 lines as moderate susceptible and only 2 lines as highly susceptible. Resistant lines were Bhathani, Bhaainagora, Hardimuri, Jhulat, Khilbhajni, Khodraphool, Lalbhog, Lamba-Asaari, Nardha, Sonpiya, Sitwadhan, Swarnagoda and Sitagora. Among 168 lines, 21 lines were moderate resistant i.e. Asamia, Chhotkadahia, Jonga, Jhona, Khilbhosni, Karhainai, Kalamdani, Khirdat, Karijiri (B), Kohraphool, Kankesal (B), Lalkisita, Mahoorinaata, Netadhan,

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Table 1. BLB Score on the basis of lesion length in cm

S.N. Variety	Delhi (kharif-2005)	Cuttack (Rabi-2005)	S.N. Variety	Delhi (kharif-2005)	Cuttack (Rabi-2005)	S.N. Variety	Delhi (kharif-2005)	Cuttack (Rabi-2005)	S.N. Variety	Delhi (kharif-2005)	Cuttack (Rabi-2005)	S.N. Variety	Delhi (kharif-2005)	Cuttack (Rabi-2005)
1. Amma Dhoka	9.5 MR	10.0MS	46. Charka Govind Bhog	10.0 MS	11.5MS	92. Jhuna	6.5 MR	7.0 MR	137. Kalam Daani Choota	7.0 MR	8.5 MR	182. Rutugora	9.0 MR	8.5MR
2. Asamia	7.0MR	7.5 MR	47. Charka Sonachur	8.0 MR	7.5 MR	93. Jhuller (B)	7.0 MR	7.5 MR	138. Kherka Kuchi (B)	9.5 MR	9.0 MR	183. Rani Kajar (A)	7.0 MR	7.0MR
3. Anjani	8.0MR	8.5 MR	48. Chhot Pansera	7.5 MR	8.5 MR	94. Jhilli	7.0 MR	7.5 MR	139. Kirdi Bot	9.5 MR	10.5MS	184. Rani Kesar	8.0 MR	8.5MR
4. Asamia (B)	6.5MR	8.5 MR	49. Charka Gora	10.5MS	11.5MS	95. Juhu (B)	8.5 MR	8.0 MR	140. Lolam Dhan	6.5 MR	7.0 MR	185. Rupsi	7.5 MR	9.0MR
5. Arjun	8.0 MR	8.5 MR	50. Charka Rani	7.5 MR	8.5 MR	96. Jhulat	3.5R	3.0 R	141. Lal Bhog	5.5R	8.0 MR	186. Ramdial	6.5 MR	7.0MR
6. Aamchhotka	7.5MR	8.0 MR	51. Charka Kherka Kuchi	8.5 MR	9.5 MR	97. Khil Bhojini	6.0R	8.5 MR	142. Lal Mansaal	8.5 MR	9.0 MR			
7. Barka Mansuri	8.5 MR	8.0 MR	52. Chotka Suman	8.5 MR	9.0 MR	98. Khilbhosni	6.5 MR	8.0 MR	143. Lal Ki Sita	6.5 MR	7.0 MR	187. Rani Kajar (B)	9.5 MR	10.5MS
8. Baraha Saal	8.0 MR	10.0 MS	53. Charka Nardha	9.0 MR	10.5MS	99. Kalam Kahi (A)	7.0 MR	9.5 MR	144. Lalbhog (B)	7.5 MR	8.5 MR	188. Raz Bhokta	6.5 MR	8.0MR
9. Bachcha Kalamdani	9.0 MR	10.0 MS	54. Doodh Kandar	7.5 MR	8.0 MR	100. Khodra Phool	5.5R	6.5 MR	145. Lal Mugdi	9.5 MR	9.0 MR	189. Rajstri	6.5 MR	7.0MR
10. Baa S Buda	9.0 MR	8.5 MR	56. Dhan Phooly	8.0 MR	7.0 MR	101. Ketki	9.0 MR	7.0 MR	146. Lamba Asari	1.6R	1.7R	190. Sirhanthi	6.5 MR	8.0MR
11. Barka Dhusri	7.5 MR	7.5 MR	57. Dhusri Safed	15.5S	12.0MS	102. Karmu Saal	9.0 MR	7.0 MR	147. Lal Jari	7.5 MR	8.5 MR	191. Son Piya	6.0 R	6.5MR
12. Bagh Panjaar	7.0 MR	7.5 MR	58. Dudha Raas	8.0 MR	9.5 MR	103. Kala Zira	7.5 MR	7.0 MR	148. Lal Dhani Barka	7.0 MR	8.5 MR	192. Sur Guja	7.5 MR	8.0MR
13. Badshah Bhog	9.0 MR	8.5 MR	59. Dhusria	7.0 MR	8.0 MR	104. Khir Beej	7.0 MR	7.0 MR	149. Lahi	8.5 MR	10.5MS	193. Sona Mati	12.5MS	11.0MS
14. Barka Swarna	10.0 MS	9.0 MR	60. Dudh Kobi	8.5 MR	9.0 MR	105. Kankesal	7.5 MR	8.0 MR	150. Lal Moti	8.5 MR	9.5 MR	194. Sira Hatti	8.5 MR	7.5MR
15. Bhamjani	7.0 MR	7.5 MR	61. Dahia (A)	7.5 MR	8.0 MR	106. Kherka Kuchi (A)	9.0 MR	8.5 MR	151. Miri Mitti	9.0 MR	7.0 MR	195. Soma	9.5 MR	8.0MR
16. Boka Dhan	11.0 MS	10.5MS	62. Dahia (B)	7.0 MR	7.5 MR	107. Khutuwa	9.0 MR	8.5 MR	152. Maina Thori	8.0 MR	7.0 MR	196. Sonagoti	9.0 MR	10.2MS
17. Bhojini	10.0MS	8.5 MR	63. Daani Gora	9.0 MR	9.5 MR	108. Kala Parwat	8.0 MR	10.0MS	153. Makar Kalma	7.0 MR	7.5 MR	197. Sona Par	9.0 MR	8.5MR
18. Budhnu Nanhia	9.0 MR	10.5 MR	64. Dudh Kandar (B)	8.5 MR	9.5 MR	109. Kera Kandhi	7.0 MR	7.5 MR	154. Mango Sar (A)	8.0 MR	7.5 MR	198. Sonachur	8.5 MR	8.0MR
19. Bhadwa Kalmndani	9.0 MR	9.5 MR	65. Dudhia	8.5 MR	9.5 MR	110. Karmi Dhan	7.0 MR	8.0 MR	155. Manjhla-Natta (A)	8.5 MR	9.0MR	199. Sitwa Dhan	2.2R	1.35R
20. Bara Sitwa	8.5 MR	9.0 MR	66. Dehati Gora (A)	9.0 MR	10.5MS	111. Kadwadhan	11.0 MS	9.0 MR	156. Maiya Dulari	8.0 MR	7.5MR	200. Suga Thor	9.0 MR	10.5MS
21. Bas Mansuri	15.5S	12.5MS	67. Dehati Gora (B)	9.5 MR	10.0MS	112. Karhaini	6.5 MR	8.0 MR	157. Mehra Dhan	10.0MS	8.5MR	201. Sir Pathi	14.5MS	13.5MS
22. Barka Tilasaar	9.0 MR	9.5 MR	68. Ejaan	7.5 MR	8.5 MR	113. Kalam Daani	6.5 MR	6.5 MR	158. Mango Sar (B)	9.0 MR	11.5MS	202. Sonam	6.5 MR	7.0MR
23. Budhnu	10.0MS	9.5 MR	69. Futua	9.5 MR	10.5MS	114. Kherka Kuchi (B)	7.0 MR	7.0 MR	159. Manjhla-Natta (B)	9.5 MR	8.5MR	203. Suleet	9.0 MR	8.5MR
24. Bala Dhusri	7.5 MR	8.5 MR	70. Gutuwa	10.5 MS	10.0MS	115. Kalam Kathi (B)	7.5 MR	7.5 MR	160. Motka-Dahia	8.5 MR	8.0MR	204. Songa-Bas	8.0 MR	10.0MS
25. Barka Sanam	7.5 MR	8.5 MR	71. Carib Saal	11.5 MS	10.5MS	116. Kadwa Dhan (B)	7.0MR	8.0 MR	161. Mahi Dhan	7.5 MR	8.5MR	205. Sita	7.5 MR	8.5MR
26. Badya	10.0MS	9.5 MR	72. Gada Jhadi	8.5 MR	7.5 MR	117. Kanak	8.0 MR	7.5 MR	162. Mahsoori Natta	6.5 MR	6.0R	206. Sonachurlal	10.6MS	11.5MS
27. Bhathani	2.1R	2.0R	73. Gudna	8.0 MR	8.5 MR	118. Kanas Chaapa	10.0MS	8.5 MR	163. Mehuri	7.5 MR	8.5MR	207. Sama	6.5 MR	7.5MR
28. Bala Joga	8.5 MR	8.0 MR	74. Guda	9.5 MR	10.5MS	119. Katika	9.0 MR	8.5 MR	164. Nanihya (A)	8.0 MR	8.5MR	208. Sathi	8.5 MR	9.5MR
29. Bala Gora	8.5 MR	9.0 MR	75. Carib Saal (B)	8.5 MR	9.5 MR	120. Kanak (B)	15.5S	12.0MS	165. Nanihya (B)	9.5 MR	8.0MR	209. Sanamdhan	7.0 MR	8.5MR

Contd.

Table 1. Contd.

S.N.	Variety	Delhi (kharif-2005)	Cuttack (Rabi-2005)	S.N.	Variety	Delhi (kharif-2005)	Cuttack (Rabi-2005)	S.N.	Variety	Delhi (kharif-2005)	Cuttack (Rabi-2005)	S.N.	Variety	Delhi (kharif-2005)	Cuttack (Rabi-2005)
30.	Bas 370	8.0 MR	10.0MS	76.	Has Kalma (A)	13.0MS	12.0MS	121.	Karhaini (B)	6.5 MR	7.0MS	166.	Naditi Kaur	7.5 MR	8.5MR
31.	Burah Dhan	9.5 MR	10.5MS	77.	Has Kalma (B)	8.5MR	9.0 MR	122.	Karjiri	3.5R	3.0R	167.	Nardha	6.0R	8.5MR
32.	Barka Kalma	8.0 MR	9.0 MR	78.	Hazari Mahak	6.5 MR	7.0 MR	123.	Khirdhat	6.5 MR	7.0MR	168.	Nambri Dhan (A)	10.0 MS	11.5MS
33.	Banphool (A)	8.0 MR	9.5 MR	79.	Hardi Muri	1.25R	2.3R	124.	Khutura	10.0MS	9.5 MR	169.	Neta-Dhan	6.5 MR	8.5MR
34.	Banphool (B)	9.0 MR	9.5 MR	80.	Hans Kalma (A)	7.5 MR	10.0MS	125.	Kohra Phool	6.5 MR	6.0R	170.	Nanka-Dhusri	7.5 MR	8.5MR
35.	Bahar	11.5 MS	12.5MS	81.	Hardi Murlilal	9.5 MR	10.5MS	126.	Kanak (B)	9.0 MR	10.5MS	171.	Nanhiya Nanka	7.5 MR	8.5MR
36.	Barka Dhan	8.0 MR	8.5 MR	82.	Hans Kalma (B)	8.0 MR	9.5 MR	127.	Kanke Saal (B)	6.5 MR	8.0 MR	172.	Nardha-Ashoka	8.0 MR	10.5MS
37.	Bas Kuchi	8.5 MR	9.5 MR	83.	Hazarek	7.5 MR	8.5 MR	128.	Karhaini Chhota	9.0 MR	10.2MS	173.	Nambri-Dhan (B)	6.5 MR	7.5MR
38.	Charin Lukia	8.0 MR	8.5 MR	84.	Is Kalma	7.5 MR	10.5MS	129.	Khira Dhat (B)	6.5 MR	6.0R	174.	01 Hajari-01	9.5 MR	10.5MS
39.	Chhotka Sitwa	7.0 MR	6.5 MR	85.	I Jun	10.0MS	9.5 MR	130.	Khera	9.5 MR	8.5 MR	175.	Pusa Basmati-1	11.0MS	11.5MS
40.	Chhotka Pansala	7.0 MR	7.5 MR	86.	Jihul	7.0 MR	6.5 MR	131.	Konhra Phool	10.5MS	11.5MS	176.	Pusa -2-21	7.0 MR	6.5MR
41.	Chhotka Dahia	6.5 MR	8.0 MR	87.	Jhinga Saal	8.5 MR	9.0 MR	132.	Kodowa	8.5 MR	9.0 MR	177.	Pala Parbat	10.2MS	9.0MR
42.	Chaanagora	6.0R	8.0 MR	88.	Jonga	6.5 MR	8.0 MR	133.	Kala Basmati	8.0 MR	8.5 MR	178.	Panch -Saala	8.5 MR	8.0MR
43.	Chairai Nerhi (A)	10.0MS	11.0MS	89.	Jhuller (A)	8.5 MR	8.0 MR	134.	Kalam Daani (B)	8.0 MR	9.0 MR	179.	Panjhali	6.5 MR	8.5MR
44.	Chairai Nerhi (B)	8.5 MR	7.0 MR	90.	Jagan Nath	6.5 MR	9.0 MR	135.	Karanga	9.0 MR	10.5MS	180.	Ratgoli	7.5 MR	8.0MR
45.	Charka Nanka	9.5 MR	9.0 MR	91.	Juhu (A)	7.5 MR	8.5 MR	136.	Karanga (B)	11.5MS	12.5MS	181.	Roopasari	7.0 MR	7.5MR

Nambridhan (B), Kanjhali, Ramdilal, Razbhokata, Rajasri, Sonam, Sarna and Sugandha. 21 lines were closer to resistant which is very near to resistant lesion length only 6.5 cm while on other hand Ammadhoka, Burahdhan, Chrkananka, both Dehatigora, A and B, Futua, Guda, Hardimurilal, Kherka, Kharkakuchi (B), Idibot, Lalmugudi, Manjhlanaata (B), Nanhia (B), 01Hajari, Ranikajjar (B), Tinthoka, near to MS with lesion length (9.5) cm.

A set tested at CRRI Cuttack in rabi-2007 with 5th isolates from Kaul, revealed that 10 lines were in resistant group, 151 were in MR group, and 48 were in MS group. Generally, all lines show more lesion length in Cuttack compared to Delhi except few, which indicate Cuttack is more favourable to Delhi for bacterial leaf blight.

Bhatani, Hardimuri, Jhulat, Karijiri (B), Lamba-asari, Mahsoori-Natta, Swarna-Gora, Sita-Gora, Sitwa-Dhan, Kohra-Phool, show high degree of resistance and except Kohra-Phool and Mahsoori-Natta, rest of the 8 lines show very high degree of resistant at both places Delhi and Cuttack. While Mahsoori-Natta is resistant in Cuttack but MR in Delhi and similarly Kohra-Phool is resistant in Cuttack but MR in Delhi with lesion length 6.5 cm.

On another side, 6 lines show resistance in Delhi like Chaaina-Gora, Khilbhojni, Khodre-Phool, Lal-bhog, Nardha, Sonpiya but they are MR in Cuttack. Chhotka-Sitwa, Sonpiya, Jlabasmati are minimum lesion length in MR group. The 19 lines namely Bhadwa Kalmdani, Barka Tilasaar, Budhnu, Badya, Banfool (A), Banfool (B), Bas-Kuchi, Charka-Khereka Kuchi, Dudharaaes, Dudhkandar (B), Dudhia, Garibsaal (B), Hanskalma (B),

1 Jun, Kalamkathi Khutura, Lalmoti, Thubka, Tulsiketki (B) are with lesion length and put in moderate resistance group and are very near to moderate susceptible group. Amma-Dhoka, Barahsaal, Bachcha-Kalamdani. Basmati 370, DehatiI-Gora (B), Gutuwa, Hans-Kalma, Kala-Parwat, Tinthoka are minimum lesion length in moderate susceptible group. Line Sir Phathi shows maximum lesion length at CRRI Cuttack (Table 1).

Eight lines Bhatani, Hardimuri, Jhulat, Karijiri (B), Lamba-asari, Swarna-Gora, Sita-Gora, Sitwa-Dhan were highly resistant in both places IARI Delhi and CRRI Cuttack. These lines were tested in next season Kharif-2007 in both of stages (Nursery and in Planted Field) and were found highly resistant so these lines can be used in breeding programs to develop bacterial leaf blight resistant variety.

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Collection and Characterisation of Field Bean [*Lablab purpureus* (L.) Sweet var. *pupureus*]

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Field bean is an ancient domesticate widely cultivated in many tropical and subtropical countries. Despite its wide distribution, adaptability and diversity, it is still considered a neglected crop and underutilised. To understand the available genetic variability and its agronomic potential for future crop improvement, an effort was made to study the variability present in field bean by collecting landraces from farmers. Two exploration and collection missions were conducted resulting in collection of 66 accessions. Among them, 47 accessions were characterised and extensive variability was observed in length, width, thickness and colour of seeds, pod colour, pod curvature, seed shape, pod length and number of pods per plant. The estimation of genotypic co-efficient of variation, phenotypic co-efficient of variation, heritability and genetic advance as % of mean revealed that leaf length, pedicel length, number of primary branches, internode length, pod length, pod width, pod yield/plant, number of pods/plant and 100 seed weight are governed by additive gene action and can be of help in direct selection from phenotypic performance. Ten lines were found potential which need to be evaluated further under multi-locations.

Key Words: Collection, Characterisation, Field bean, *Lablab purpureus* var. *pupureus*, Variability, Landraces, Heritability and Genetic advance

Introduction

Field bean or *Lablab purpureus* (L.) Sweet previously known as *Dolichos lablab* (L.), is cultivated in different parts of the world. It is an ancient domesticate widely distributed in many tropical and subtropical countries where it has become naturalised (Purseglove 1968). In South and Central America, East and West Indies, Asia, China and India, field bean is grown as an annual or as a short-lived perennial (Whyte *et al.*, 1953). In these areas, the seed and immature pods are used for human food while the herbage is used as green manure, for erosion control, and as a feed supplement for cattle grazing mature pasture in the dry season (Hendricksen and Minson, 1985). It is used as vegetable (green pods and seeds) and grain legume (Hanelt, 2001). The vegetable type is known as *Lablab purpureus* var. *pupureus* Verdc. and the pulse type as *L. purpureus* var. *lignosus* (Prain) Kumari. The pulse type is called motcha in Tamil Nadu (Nair and Henry, 1983). The field bean is undoubtedly wild in India and also in Java (Hooker, 1879; Candolle, 1959) and was introduced into Africa from South-East Asia during the eighth century (Kay, 1979). It still remains as an important, but minor, crop in many of these regions. In general, tropical legumes tend to be higher in crude lignin and protein and lower in cell wall than tropical grasses (Van Soest, 1994). With an average crude protein of 17% in the dry matter, field bean is slightly above average when compared to other

tropical legumes and has considerably more protein than tropical grasses (Norton, 1982). The average of 28% crude fibre in dry matter of field bean ranks less than the majority of tropical legumes and grasses (Norton, 1982). In terms of dry matter digestibility, field bean ranks well compared to both tropical grasses and legumes (Minson and Wilson, 1980). Despite its wide distribution in the tropics, adaptability and diversity, field bean is still considered a neglected and underutilised crop. However, its use as a vegetable, pulse and/or forage crop in the tropical regions with humid to semi-arid climates has resulted in reassessing the potentiality for cultivation in tropical farming systems. For this, an understanding of available genetic variability and its agronomic potential for future crop improvement is essential. Hence an effort was made to collect and study the variability present in field bean.

Materials and Methods

Two exploration and collection missions were conducted to the central and the northern region of Tamil Nadu in April and May 2004 respectively. The physiographic condition of the area surveyed was plains, consisting of red, black or brown loamy soil. The sampling strategy followed was selection of an individual plant from among a population for collection of mature pods. Among the 66 collections, characterisation for five seed characters namely seed length, seed width, seed thickness, 100 seed weight and

seed colour was done in *in situ* condition at the time of collection in 47 accessions. The data was recorded on five seeds/accession. Field evaluation was also done in these 47 accessions in an augmented block design along with 4 check varieties namely RND-1, CO-12, CO-13, DL-40 and DL/6-1 in 5 blocks at Department of Olericulture, College of Horticulture, Kerala Agricultural University. The experiment was conducted in *rabi* 2004-05 and three plants per accession were planted and all plants were taken into consideration for scoring. A total of 22 characters (11 quantitative and 11 qualitative) was recorded. The minimal descriptor of field bean developed by National Bureau Plant Genetic Resources was used. Data were averaged and analysed for standard statistical procedure for calculating range, mean and standard deviation followed for estimating the genotypic and phenotypic coefficient of variation (Burton, 1952), heritability (Hanson *et al.*, 1956) and genetic advance (Johnson *et al.*, 1955). Frequency distribution of each qualitative character was computed.

Results and Discussions

Exploration and Collection

The two explorations and collection missions executed resulted in the collection of 66 accessions. A total of 37 collection sites in 10 districts of Tamil Nadu was covered (Figure 1). The accessions collected district wise and the landraces collected are given in Table 1. In general, farmers grow this crop in pandals *i.e.*, pole type in the backyard or kitchen garden. The unique landraces of vegetable type collected in Tamil Nadu included *aattukombu avarai* (IC426964) from Villupuram district with twisted pods

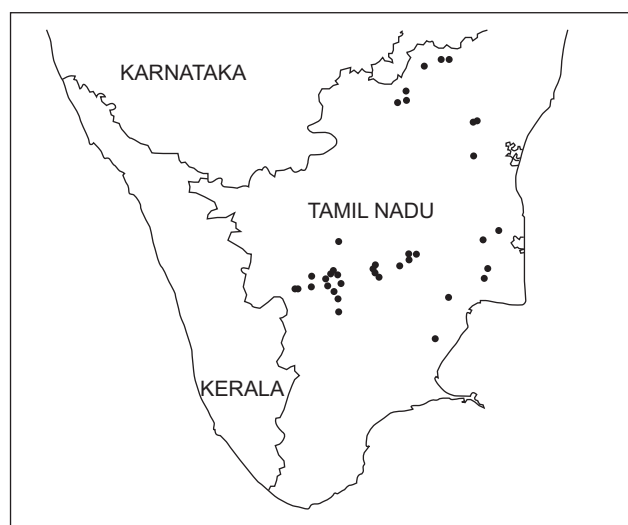


Fig. 1: Collection Sites in Tamil Nadu

Table 1. Cultivars collected from Tamil Nadu

Districts	Accessions (No.)	Cultivar names
Dindigul	27	<i>belt avarai</i> , <i>koli avarai</i> , <i>pacha avarai</i> , <i>pattai avarai</i>
Karur	5	<i>sevavarai</i> , <i>kanu avarai</i> , <i>neela avarai</i>
Krishnagiri	1	<i>thotta avarai</i>
Madurai	2	<i>aniseviavarai</i> , <i>pattai Avarai</i>
Pudukkottai	1	<i>pachai Avarai</i>
Thanjavur	1	<i>avarai</i>
Thiruvavur	3	<i>avarai</i>
Tiruchirappalli	13	<i>avarai</i> , <i>belt avarai</i> , <i>pachavarai</i> , <i>koli avarai</i> , <i>kanu avarai</i> , <i>patta avarai</i> , <i>ayarimai belt avarai</i>
Vellore	7	<i>veettu avarai</i> , <i>koli avarai</i> , <i>nattu avarai</i>
Villupuram	6	<i>pacha avarai</i> , <i>vella avarai</i> , <i>aattu kombuavarai</i>

like a horn of sheep; *kozhiavarai* (IC426951, 426968, 426978, 426983, 426983, 426985, 426989 and 426993) from Dindigul and Tiruchirappalli districts with thin pods that resembled the leg of a chicken; *aniseviavarai* (IC427443) from Madurai district with broad pods (width of >4.0 cm) that resembled the ear of an elephant. The market attraction goes to *pattaiavarai* (IC427425) with completely purple colored pods and pod length ranging from 23.0-26.0 cm with high yield (60kg/plant).

In situ Characterisation of Seed

Extensive variability was found in seed length, seed width, seed thickness, 100 seed weight and color. The range, mean, CV (%) and SD are given in Table 2. The seed color varied from black, black mottled with brown, greenish brown, reddish brown, reddish brown with light brown mottling, brown, shiny reddish brown, cream with dark brown mottling and maroon. The seed length varied from 9.16 to 12.17 mm, width from 7.07 to 10.81 mm, thickness from 4.45 to 7.59 mm and 100 seed weight from 16 to 50 g.

Ex situ Morphological Characterisation

Forty-seven accessions were characterized on the basis of distinguishable phenological and agro-morphological characters. Among the 11 qualitative characters studied, all the accessions were found to be climbing type with stalked inflorescence and were photosensitive. The frequency distribution for rest of the characters is given in Table 3. More variability was found in pod color, pod curvature and seed shape (Fig. 2). The analysis of variance indicated highly significant variation among the

Table 2. *In situ* characterisation of seed characters of field bean

Accessions	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	100 seed weight
Range	9.16-12.97	7.07-10.81	4.45-7.59	16-50
Mean	11.41	8.57	6.07	34.12
SD	0.81	0.73	0.68	8.23
CV (%)	7.07	8.50	11.15	24.13

Table 3. Frequency distribution of qualitative traits

Qualitative Characters	Descriptor states	Accessions (no.)	Frequency %
Stem color	3	37	78.72
	5	10	21.28
Pod color	3	3	6.38
	4	35	74.47
	5	3	6.38
Pod suture color	99	6	12.77
	2	8	17.02
	99	39	82.98
Pod shape	4	37	78.72
	5	10	21.28
Pod curvature	1	26	55.32
	2	6	12.77
	99	15	31.91
Pod surface	1	34	72.34
	2	13	27.66
Seed shape	1	12	25.53
	2	14	29.79
	3	21	44.68
Leaf vein color	2	37	78.72
	3	10	21.28

genotypes for all the quantitative characters except pod width and pod weight/plant (Table 4). The variability may be due to genetic constitution of the materials as well as environmental influence. The development of an effective plant breeding programme is dependent on the existence of genetic variability. The efficiency of selection depends upon the magnitude of genetic variability present in the plant population. The estimates of mean, range, phenotypic co-efficient of variation (PCV) and genotypic co-efficient of variation (GCV), heritability (h^2) and genetic advance (GA) as % of mean are presented in Table 5. Maximum range was found for pod yield per plant (kg) (0.049-6.05) and minimum range for number of primary branches per plant (2-6). High magnitude of PCV than GCV for all the characters indicated considerable influence of environment on the expression of the characters. Maximum PCV and GCV was observed for number of pods/plant and pod yield/plant. Moderate PCV and GCV for leaf length, pedicel length, pod length and pod width. All the other characters exhibited low GCV and PCV.

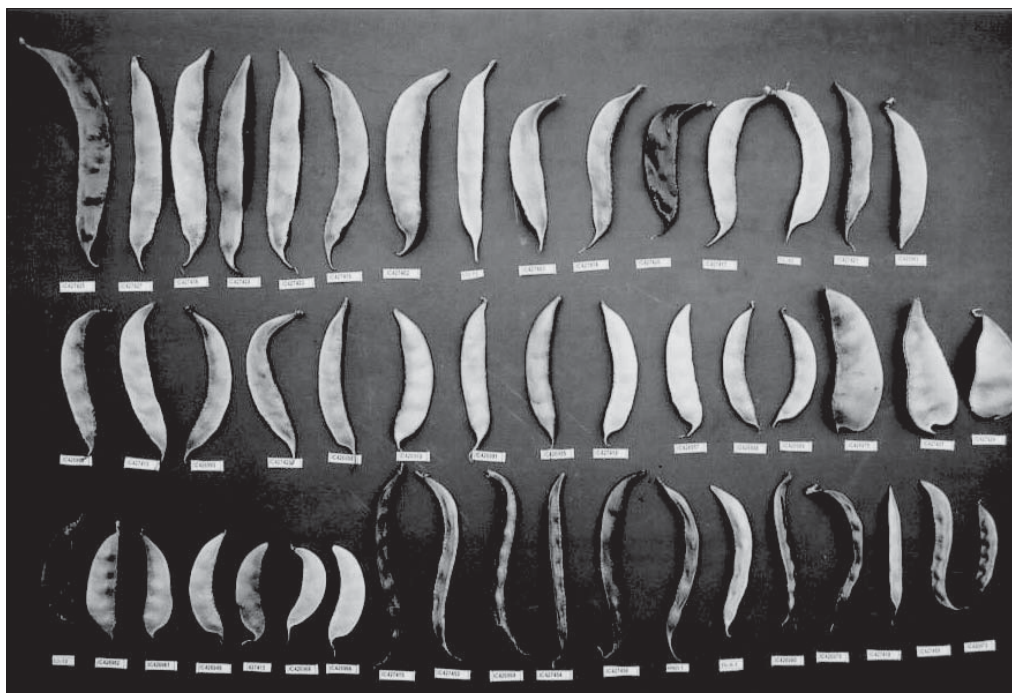
**Fig. 2: Variability in pod characters**

Table 4. Analysis of variance of quantitative traits

Characters	Mean sum of squares			CD (0.05%)
	Rows	Columns	Error	
Leaf length (cm)	10.42**	0.36	0.75	2.31
Leaf breadth (cm)	7.66**	0.47	1.03	2.71
Pedicle length (cm)	62.00**	5.09	3.22	4.79
No. of primary branches	2.11**	0.05	0.05	0.57
Internode length (cm)	20.74**	1.83	1.74	3.52
Pod length (cm)	30.35**	0.48	0.63	2.12
Pod width (cm)	1.21	0.01	0.03	0.47
Pod yield per plant (g)	2253422.08**	25233.87	26632.41	435.72
No. of pods per plant	335558.55**	3265.65	4665.98	182.38
Pod weight (g)	0.35	0.08	0.11	0.88
100 seed weight (g)	167.39**	4.31	3.60	5.07

Heritability values ranged from 30.38 to 94.36 % for the characters studied. The highest heritability was noted for pod yield/plant, number of pods/plant, pod length, pod width, 100-seed weight and number of primary branches. These characters have more reliable phenotypic performance and there could be more relationship between phenotypic and breeding values. This indicated that

substantial genetic improvement can be achieved for these characters. The moderate level of heritability was noted in leaf breadth and internode length and low heritability for pod weight. Heritability only denotes the percentage of effectiveness with which the selection can be based on the phenotypic performance. In order to assess the genetic progress, genetic gain should be measured along with heritability. The value of expected genetic advance was high for the characters like leaf length, pedicle length, number of primary branches, internode length, pod length, pod width, pod yield/plant, number of pods/plant and 100 seed weight and this suggested that these characters are governed by additive gene action and selection will be rewarding for improvement of these traits. Moderate and low genetic advance was recorded for leaf breadth and 100 seed weight. For more reliable conclusion, estimates of heritability and genetic advance should be considered together, which is more useful than heritability alone (Singh and Narayan, 1993). High heritability combined with high genetic advance was recorded for characters like leaf length, pedicle length, number of primary branches, internode length, pod length, pod width, pod yield/plant, number of pods/plant and 100 seed weight and moderate heritability and genetic advance was seen

Table 5. Range, mean and co-efficient of variations of quantitative characters

Character	Mean	GCV (%)	PCV (%)	Heritability (h ²)	Genetic advance as % of mean
Leaf length (cm)	10.82	12.85	24.85	72.06	22.45
Leaf breadth (cm)	9.92	11.63	15.09	56.36	17.94
Pedicle length (cm)	15.36	22.33	25.76	78.53	40.88
No. of primary branches	3.95	16.2	17.22	89.18	31.62
Internode length (cm)	14.24	13.69	16.5	68.59	23.34
Pod length (cm)	11.35	21.49	22.56	90.04	42.11
Pod width (cm)	2.15	22.79	24.19	88.88	44.19
Pod yield per plant (kg)	1.26	52.89	54.47	94.36	112.18
No. of pods per plant	436.44	59.36	61.38	93.49	118.24
Pod weight (g)	3.42	6.41	11.62	30.38	7.28
100 seed weight (g)	30.32	18.84	25.02	88.33	36.44

Table 6. Superior lines identified on the basis of quantitative characters

Character	Accessions (no.)
Pod length (cm) > 14.0 cm	IC427424A, 427427, 427423, 427436, 427463, 427456, 427421, 427428, 427413A, 427424B
Pod width (cm)	IC427424, 427457, 427437, 427445, 426975, 426976
Pod yield/plant (g)	IC426983, 426970B, 427413C, 426980, 426960, 426961, 427462, 427419, 426993, 427421
No. of pods per plant	IC426983, 427413C, 426980, 426970B, 426960, 427419
Pod weight (g)	IC427462, 426959, 427457, 27445, 426958, 427427, 427424A, 427436, 426968, 427428A, 427463, 427424, 427423

in leaf breadth. Hence these characters are governed by additive gene action and can be of help in direct selection from phenotypic performance. On the basis of evaluation of germplasm for various traits, accessions which were found superior were selected and presented in Table 6. IC426983, 426970B, 427413C, 426980, 426960, 426961, 427462, 427419, 426993 and 427421 were potential lines which need to be evaluated further under multi-location testing.

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Genetic Divergence of Germplasm Accessions in Potato (*Solanum tuberosum* subsp. *tuberosum*)

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One hundred ninety five accessions of tuberosum group of potato were evaluated for nine distinct characters during three consecutive autumn crop seasons to assess the genetic divergence based on Non-hierarchical Euclidean distances for the identification of genetically diverse and agronomically superior accessions which may generate putative transgressive segregates on hybridization. Based on first five principle components, namely plant height, number of tubers, tuber yield, and number of stems and germination, which accounted for 97.67% of the variation, 195 potato accessions were grouped into 8 well distinct clusters. Geographical diversity was not related to genetic diversity. Twenty genetically diverse and agronomically promising genotypes were identified, which may be used in crossing programme to obtain segregates with commercially desirable attributes for development of potato varieties for sub-tropical plains of India.

Key Words: Potato, Germplasm accessions, Genetic divergence, Cluster distance

Introduction

Potato is a highly heterozygous crop and increase in its heterozygosity results in hybrid vigour (Tarn and Tai, 1977). Crosses involving genetically diverse parents are likely to produce high heterotic effects (Gopal and Minocha 1997, Luthra *et al.*, 2005). Analysis of genetic diversity in germplasm collection facilitates reliable classification of accessions and identification of core accession with possible utility for specific requirements (Mohammadi and Prasanna, 2003). Multivariate analysis based on Mahalanobis D² statistics has limitations for classifying huge germplasms for several characters (Arunachalam, 1981). The use of Non- hierarchical Euclidean cluster analysis to overcome these limitations has been suggested by Beale (1969). In potato, limited information on genetic divergence is available (Pandey and Gupta, 1995; Kumar and Kang, 2000; Sandhu *et al.*, 2001; Singh, *et al.*, 2004; Luthra *et al.*, 2005) for subtropical plains which account nearly 85-90% of total production of the country. For meeting the varietal requirement of such vast area, genetically diverse parents with high tuber yield as well as desirable tuber attributes are required for making desirable cross combinations to obtain superior progenies from segregates. Hence, the present investigation was conducted to determine genetic divergence and identify the genetically diverse and agronomically superior germplasm accessions for use as parent in developing potato varieties.

Materials and Methods

The experimental material comprising 195 potato germplasm accessions (*Solanum tuberosum* subsp. *tuberosum*) was grown in RBD with three replications during autumn crop seasons of 1999-2000, 2000-2001 and 2001-2002 at CPRI Campus Modipuram, Meerut (29°N and 76°E; 222 msl). Fifteen tubers of each accession in single row of 3m planted at inter- and intra-row spacing of 60 cm and 20 cm respectively, represented each replication. Recommended cultural practices were followed. The trial was harvested at 90 days after planting and the observations were recorded on five randomly selected plants on nine characters (Table 3). Mean values were used for principal component analysis and the principal component scores were used for Non-hierarchical Euclidean cluster analysis (Beale, 1969; Spark, 1973). Statistical package SPARI was used for these computations. The appropriate number of clusters to group 195 accessions was determined following the 'Sequential F-ratio tests' (Rao, 1952).

Results and Discussion

Significant differences were found among the genotypes for all the characters studied indicating the availability of considerable diversity in the germplasm accessions. The principal component analysis yielded 9 Eigen vectors and roots. The per cent variation explained by nine roots; 1 to 9 was 40.29, 33.64, 10.72, 8.12, 4.90, 1.48, 0.56, 0.22 and 0.08 respectively). Further, highest maximum Eigen root value (3.626) was obtained by Eigen vector 1, followed

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by 3.027, 0.965, 0.731, 0.441, 0.133, 0.050, 0.020 and 0.007, respectively for the rest of the vectors. The first five principal components calculated by using standard variables, accounted for 97.67% of total variation. The first component was measure of plant height, second number of tubers, third of tuber yield, fourth of number of stems and fifth of germination (%), as the coefficients associated with these traits have the largest magnitude. These results confirmed the earlier findings on plant height (Sandhu *et al.*, 2001; Singh *et al.*, 2004), tuber number (Kumar and Kang 2000), tuber yield (Sandhu *et al.*, 2001) with the deviation of ranking of stem number and germination contributing toward genetic divergence which may be attributed to the material used and environmental conditions.

The 195 potato accessions were grouped into 8 groups (I-VIII) based on similarity in traits. The

highest number of accessions (52) fell in cluster VI, while minimum number (12) were observed in cluster I and III (Table 1). Average distance of cluster-means from cluster centroids (intra-cluster distance; Table 2) ranged from 1.306 (cluster VII) to 2.349 (cluster VIII). The genotypes of the same cluster had little divergence from each other with respect to aggregate effect of 9 characters examined. In general, genotypes of same origin fell in different clusters indicating thereby that geographical diversity is not related to genetic diversity. Pandey and Gupta (1995) also found that 52 potato genotypes did not follow their geographical distribution and 11 resultant clusters were heterogeneous with respect to genotypes and their place of origin. Therefore, geographical diversity of the material alone would not help in selection of genetically divergent parents. In the present study four Indian potato

Table 1. Distribution of 195 potato germplasm accessions on the basis of Non-hierarchical Euclidean cluster analysis for 9 characters

Cluster No	Number of accessions	Accessions (CP numbers)
I	12	CP1242*, 1361 (Albion), 1386 (Earlaine), 1409 (Fruhmolle), 1418 (Maritta), 1424 (Pionier), 1436 (Urgenta), 1440 (BD1456c(1)), 1443 (BD237/43), 1582 (Tawa), 1586 (PI197932), 1632 (XD2)
II	27	CP1057*, 1151*, 1163*, 1207*, 1329*, 1338*, 1358 (Idaho), 1367 (B922-6), 1399 (Ari), 1401 (Barima), 1414 (Irene), 1431 (Rival), 1442 (2613b(11)), 1456 (Febricia), 1462 (Spatz), 1507 (3305(9)), 1515 (3392(1)), 1516 (3392(4)), 1524 (Roslin Sasumua), 1538 (M124-2), 1555 (Pimpernel x 1006-212), 1596 (B4808-19), 1633*, Kufri Anand, Kufri Bahar, Kufri Pukhraj, Kufri Sutlej
III	12	CP658*, 721*, 1340*, 1343 (Kardinal), 1362 (Ag14(X37)), 1365 (AG54-55(self)), 1395 (B3627-12), 1417 (Luctor), 1427 (Profijt), 1527 (2403a(1)), 1529 (2305a(10)), 1545 (Saskia)
IV	13	CP1123*, 1172*, 1225*, 1243*, 1353 (Croissement), 1379 (96-56), 1390 (B3620-1), 1421 (Pandora), 1455 (Drossel), 1466 (Konsagris), 1473 (Ulanpur), 1482 (B3352-8), 1607 (B5088-26)
V	31	CP1187*, 1213*, 1325*, 1339*, 1364 (AG29), 1374 (B3781-3), 1378 (B595-76), 1380 (B3139-24), 1385 (B929-23), 1400 (Avenir), 1403 (Bevelander), 1404 (Burmania), 1408 (Froma), 1425 (Perfect), 1428 (Record), 1453 (Amsel), 1460 (Schwalbe), 1470 (Jiiueca), 1480 (B927-3), 1487 (B3960-1), 1497 (2814a(1)), 1503 (3069(d)), 1513 (3339d(13)), 1518*, 1523 (Roslin Riviera), 1530 (2591k(3)), 1576 (PI222945), 1595 (B4808-8), 1598 (B4845-4), 1604 (B5052-7), 1616 (835a(4))
VI	52	CP1180*, 1181*, 1205*, 1218*, 1232*, 1235*, 1237*, 1275*, 1304*, 1317*, 1346 (Krirrinee), 1350 (Monak), 1355 (Rhode Island), 1373 (B35-7), 1383 (B3404-1), 1391 (B3172-13), 1392 (B3603-28), 1402 (Bea), 1415 (Kwinta), 1420 (Meerster), 1433 (Sientje), 1441 (BD-1456c(1)), 1444 (5050/23/1), 1445 (Anita), 1451 (Lori), 1457 (Fink), 1465 (Electra), 1472 (Kpaxunuper), 1474 (Duquesa), 1483 (B3556-12), 1485 (B3604-19), 1491 (BD3053-18), 1519*, 1546 (Sirtema), 1547 (Pentland Envoy), 1548 (2787e(22)), 1564 (B4829-7), 1588*, 1589 (Datura), 1590*, 1591*, 1592 (B4744-23), 1594 (B4795-3), 1597 (B4830-19), 1619*, 1626 (2070(59)), Kufri Ashoka
VII	33	CP1302*, 1308*, 1342 (Jara), 1344 (Kotnov), 1345 (Krasava), 1348 (Adina), 1352 (Blank), 1363 (AG-16(X143)), 1366 (B721-35), 1368 (B3428-20), 1382 (B3401-15), 1388 (B3626-13), 1398 (Ambassadeur), 1407 (Dore), 1412 (Herkol), 1413 (Ijesselster), 1429 (Regina), 1432 (Rode Eersteling), 1435 (Tendira), 1439*, 1448 (Elenita), 1449 (Capella), 1476 (Olalla), 1492 (VD2-21), 1539 (M136-6), 1544 (Asoka), 1566 (B4986-15), 1574 (B5132-3), 1602 (B5030-10), 1612 (Vendal)
VIII	15	CP1086*, 1175*, 1215*, 1231*, 1233*, 1310*, 1314*, 1316*, 1330*, 1351 (Walanga), 1375*, 1463 (Spika), 1475 (Goya), 1479 (B-751-119), 1615 (Craigs Royal)

Name in parenthesis are the original donor name; * Original donor name not available

Table 2. Average inter- and intra-cluster (bold) distances in potato germplasm accessions

Cluster number	I	II	III	IV	V	VI	VII	VIII
I	2.205	6.240	4.093	4.334	3.490	4.658	2.625	7.187
II		2.047	5.999	4.145	2.941	2.647	4.301	4.894
III			1.659	4.166	4.835	3.390	2.507	3.983
IV				2.259	2.979	3.177	3.193	5.295
V					1.527	2.683	2.521	5.881
VI						1.547	2.159	3.245
VII							1.306	4.631
VIII								2.349

Table 3. Cluster means for 9 characters in potato germplasm accessions

Characters	Cluster							
	I	II	III	IV	V	VI	VII	VIII
Germination (%)	96.03	97.72	96.80	86.60	95.99	98.24	97.83	99.00
Plant height (cm)	45.70	58.56	44.78	49.17	51.99	52.22	49.94	56.54
Stems/plants	3.18	3.65	4.42	3.66	3.14	4.17	3.94	5.24
Tuber yield (g/plant)	172.30	369.63	266.37	295.99	268.38	329.10	253.93	391.60
Number of tubers/plant	6.52	7.76	12.54	8.48	6.47	9.09	8.62	13.16
Average tuber weight (g)	27.16	48.89	22.34	35.77	42.61	36.97	29.79	30.38
Marketable tuber yield (g/plant)	146.88	348.33	217.72	270.11	248.61	300.51	223.63	349.65
Number of marketable tubers/plant	4.10	5.72	6.55	5.77	4.77	6.34	5.43	8.29
Marketable average tuber weight (g).	35.86	61.39	32.73	47.67	52.26	47.55	41.41	41.80

varieties namely Kufri Anand, Kufri Bahar, Kufri Pukhraj and Kufri Sutlej were grouped together in cluster II, though they were bred from parent of wide genetic base. This is because these varieties were developed with the main aim of high yield under similar agro-climatic conditions of sub-topical plains.

The maximum inter-cluster distance (7.18) was found between clusters I and VIII followed by cluster I and II (6.24), cluster II and III (5.99), cluster V and VIII (5.88), cluster IV and VIII (5.29) and cluster II and VIII (4.89). The minimum inter-cluster distance (2.50) was observed between cluster III and VII (Table 2). Superior potato accessions with highest mean values for germination (99%), number of stems (5.24), tuber yield (391.60 g/plant), number of tubers (13.16 tubers/plant), marketable tuber yield (349.650 g/plant) and number of marketable tubers (8.29 tubers/plant) fell into cluster VIII, while highest plant height (58.56 cm), average tuber weight (48.89 g) and marketable average tuber weight (61.39 g) converged to cluster II (Table 3).

The data on inter-cluster distances and mean performance of potato accessions were used to select genetically diverse and agronomically superior genotypes from 195 potato germplasm accessions. Cluster II and VIII contained genotypes which were better in respect of one or more characters and comparable with control in respect to other characters. Superior genotypes were, however, also selected from other clusters, which were widely separated from cluster II and VIII but had desirable traits. In all, 12 promising accessions were selected from cluster II (CP1207, 1329, 1367, 1515, 1538, 1555, 1596, 1633, Kufri Anand, Kufri Bahar, Kufri Pukhraj and Kufri Sutlej), one accession each from cluster III (CP1362) and cluster VI (CP1181); and 8 accessions from cluster VIII (CP1215, 1310, 1316, 1330, 1351, 1475, 1479, 1615) (Table 4). Use of these genotypes in potato breeding programme as multiple crossing among them may result in segregates with commercially desirable attributes for development of potato varieties for subtropical plains of India.

Table 4. Diverse and superior potato germplasm accessions and their tuber attributes

Genotype	Tuber Yield/plant	Number of tubers/plant	Average tuber weight (g)	Tuber characters (Skin colour, tuber shape, eye depth, flesh colour)
CP 1181	402.10**	10.59*	38.43	Yellow, oval, deep, cream
CP 1207	369.28**	5.86	64.58**	Yellow, oval, shallow, white
CP 1215	360.11**	18.85**	20.42	Yellow, round, medium, cream
CP 1310	414.77**	13.05**	31.51	White, oval, medium, light yellow
CP 1316	540.71**	15.68**	32.26	Yellow, oval, medium, yellow
CP 1329	397.43**	8.48	47.07**	Yellow, oval, shallow, cream
CP 1330	442.27**	12.45**	35.52	White, round, medium, yellow
CP 1351	424.94**	12.92**	33.14	White, oval, medium, white
CP 1362	277.58	19.21**	14.38	White, oval, shallow, cream
CP 1367	390.25**	8.20	46.60**	White, oval, shallow, white
CP 1475	386.52**	14.39**	27.48	White, oval, medium, cream
CP 1479	366.68**	14.21**	25.72	Yellow, oval, shallow, white
CP 1515	456.48**	10.31*	49.98**	White, oval, shallow, white
CP 1538	476.49**	10.27*	49.01**	White, oval, shallow, white
CP 1555	372.35**	7.78	46.62**	Yellow, oval, shallow, white
CP 1596	402.41**	8.85	45.77*	Yellow, oval, medium, light yellow
CP 1615	414.37**	11.16**	36.41	Yellow, oval, medium, yellow
CP 1633	342.17	7.12	52.23**	White, oval, shallow, light yellow
Kufri Anand	431.11**	10.24*	42.09	White, long- oval flattened, fleet, white
Kufri Bahar	389.72**	8.08	51.88**	White, oval, medium, white
Kufri Pukhraj	448.92**	8.55	49.11**	Light yellow, oval, shallow, yellow
Kufri Sutlej	414.20**	7.38	56.38**	White, oval, fleet, white
Mean	300.96	8.74	36.17	
CD at 5%	41.78	1.45	7.41	
CD at %	54.92	1.90	9.73	

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Genetic Divergence for Morpho-physiological Traits and Yield Components Associated with Drought Tolerance in Maize (*Zea mays* L.) Inbreds

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Forty-one maize inbreds were evaluated for obtaining information regarding genetic divergence through multivariate analysis using Mahalanobis' D^2 statistic for a set of 20 divergent characters. The analysis of variance revealed significant differences among the genotypes for all the traits except canopy air temperature difference. The D^2 analysis revealed that genotypes exhibited considerable diversity and were grouped into nine clusters, where cluster I and V accommodated maximum and equal numbers of entries (11 each). The inter-cluster distance was highest between cluster III and cluster VIII followed by cluster III and VIII, II and VII. The maximum contribution towards genetic divergence was observed by 100 kernel weight followed by dry stover yield, relative water content, proline content and grain yield. Based on Tocher's clustering pattern of the genotypes, eight inbreds were suggested for inclusion into hybridization for obtaining high heterotic crosses.

Key Words: D^2 Statistic, Drought tolerance, Genetic Divergence, Maize, *Zea mays*

Introduction

Maize is one of the most important cereal crop in the world; it finds third important position in India after rice and wheat with enormous role in food and nutritional security. Drought is one of the major causes of yield losses in maize in the world. The risk of drought is particularly high in rainfed maize cultivation. Drought that coincides with the flowering period can cause serious yield instability at the farm level, because it allows no opportunity for farmers to replant or otherwise compensate for loss of yield. Even a single irrigation omission during one of the sensitive growth stages, may cause a 30-40% grain yield loss (Cakir, 2004). In order to develop genotypes with desirable traits, the breeders choose genetically distant parents, as genetic diversity plays an important role in plant breeding because hybrids derived from the lines of diverse origin display a greater heterosis than those between closely related strains. Estimation of genetic divergence also allows breeders to eliminate some parents in downsizing the core collections maintained and concentrate their efforts in a smaller number of hybrid combinations (Fuzzato *et al.*, 2002). Several methods have been developed to study the extent of genetic divergence in the genotypes among which Mahalanobis' generalized distance (D^2) (Mahalanobis, 1936) is frequently used. D^2 analysis is a useful tool in quantifying the degree of divergence between biological populations at genotypic level and to assess relative contribution of different components to the total divergence both the inter- and intra-cluster levels. In order to utilize the material for further improvement,

it is imperative to know the extent of diversity present among the inbreds. Keeping this in view, the present investigation was carried out to know the magnitude of diversity present in these introduced and locally developed inbreds for morpho-physiological and yield components related to drought tolerance and to select diverse parents with an aim to obtain heterotic crosses and wide array of combinations.

Material and Methods

The material for the present investigation consisted of 41 maize inbreds obtained from DMR, New Delhi; PAU, Ludhiana; CIMMYT, Mexico and SKUAST-Jammu (Table 1). The experiment was conducted under three environments (irrigated, limited irrigation and rainfed) during *kharif* 2005 at Research Farm of Division of Plant Breeding and Genetics, SKUAST-J, Chatha, Jammu. Each genotype was sown in a two row plot of 5 m length spaced at 60 cm with interplant distance of 25 cm. The experiment was laid in Randomized Block Design with three replications. The recommended package and practices were followed to raise a good crop. Observations were recorded on twenty characters. In each entry five plants were randomly selected and utilized to collect observations on plant height, ear height, leaves per plant, ears per plant, ear length, ear girth, kernel rows per ear, kernels per row, 100-kernel weight, grain yield per plant, dry stover yield per plant and biological yield per plant. Data for days to 50 per cent pollen shed, days to 50 per cent silk, anthesis-silking interval, days to maturity, number of barren plants per plot and canopy-air temperature

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Table 1. List of maize inbreds, pedigree and source

S.No.	Name	Pedigree	Source
1	LM5	(Tux Pool C2IC2-5-1-1-2-2-3-3-1 F)	PAU, Ludhiana
2	LM6	(MS Pool C2IC2-5-1-2-1-1-1-2-1-1 F)	PAU, Ludhiana
3	LM9	(MS Pool C2IC2-11-2-2-1-1-1-1-#-FSb-1-#-#)	PAU, Ludhiana
4	LM10	(Pop28TSR (S2)-13-#-#-6F-#-1-3-1-#-I-#-#-#)	PAU, Ludhiana
5	LM11	Sw 1-26-1-1-1-1-1-1-#-#	PAU, Ludhiana
6	LM12	J54.M.17 #-21-2-3-2-2-1-1⊗b	PAU, Ludhiana
7	CM105	Peru 330-#-#329c-1-f-#	DMR, New Delhi
8	CM111	Cuba 342-2-f-#-#	DMR, New Delhi
9	CM115	Eto 182-1-2-f-f-#-A bulk-#	DMR, New Delhi
10	CM116	Puerto Rico Gr-I-A 116-f-#	DMR, New Delhi
11	CM117	Cuba 11J-A46-f-#	DMR, New Delhi
12	CM121	A derivative from A dec-A257	DMR, New Delhi
13	CM122	(J 61 7-61-1-1-1-1-1 #)	DMR, New Delhi
14	CM125	(H 104-15-1-1-3g-1-1#)	DMR, New Delhi
15	CM132	B57E#-1-3-#-3-#-⊗-#-f-##	DMR, New Delhi
16	CM135	IPA-3-6-10-3-#-⊗-⊗-#-⊗	DMR, New Delhi
17	CM137	IPA-3-7-f-⊗-#-⊗-⊗ derived from DMR	DMR, New Delhi
18	CM138	IPA-21-10-f-#-⊗ derivative of AD609	DMR, New Delhi
19	CM139	{(Tarun x MSiY-63-1g-2-1-1-2##-1-1-1-1#FS2###)}	PAU, Ludhiana
20	CM140	(J617-61-1-1-1-f-FS5-1-1####)	PAU, Ludhiana
21	CM142	IPA-34-62-f-1-1-1-1	DMR, New Delhi
22	CM143	(JS2 x J3022) H.S.43-2-1-1-2-#FS.2-#-#-1-#-b-#	PAU, Ludhiana
23	CM144	SE533: (Tarun x MSI)-Y63-ig-2-1-1-#-#-3-1-2-1-1-F ₁ s bulk-#-#-#	PAU, Ludhiana
24	CM207	Fla3H94-f-#-#2-1-1-b	DMR, New Delhi
25	CM 213	Fla6H13-f-#-#-1-1-1-1	DMR, New Delhi
26	Y-500	Pool-500# 2-2-1-1-1-1-1⊗b	SKUAST-JAMMU
27	Y-501	Pool-501# 5-3-2-1-1-1-1⊗b	SKUAST-JAMMU
28	CML228	EV88SUWAN1-SR(BC5)-201-1-1-b-b	CIMMYT, Mexico
29	CL02443	Pob24STEC1HC45-1-2-3-3-2-BB-f	CIMMYT, Mexico
30	Ib6105	Pob45-1-2-1-1-1-1-1⊗b	SKUAST-Jammu
31	Ib6118	C6-5-1-2-1-1-1-1⊗b	SKUAST-Jammu
32	Ib6135	Pob45-5-2-1-1-1-1-1⊗b	SKUAST-Jammu
33	Ib6140	Pob33-3-2-1-1-1-1-1⊗b	SKUAST-Jammu
34	Ib6144	Pob33-7-2-1-1-1-1-1⊗b	SKUAST-Jammu
35	Ib3010	Super1-1-2-1-1-1-1-1⊗b	SKUAST-Jammu
36	PMS-14	Super1-14-2-1-1-1-1-1⊗b	SKUAST-Jammu
37	M4-4	MRTxSuper1#-4-4-1-1-1-1-1⊗b	SKUAST-Jammu
38	M7-7	MRTxSuper1#7-7-1-1-1-1-1⊗b	SKUAST-Jammu
39	M8-3	MRTxSuper1#8-3-1-1-1-1-1⊗b	SKUAST-Jammu
40	M13-8	MRTxSuper1#13-8-1-1-1-1-1⊗b	SKUAST-Jammu
41	M16-5	MRTxSuper1#16-10-1-1-1-1-1⊗b	SKUAST-Jammu

difference were recorded on plot basis. Free proline content from leaf samples was estimated following the method of Bates *et al.* (1973). Relative water content was estimated using formula: $RWC (\%) = [(F_w - D_w) / (T_w - D_w)] \times 100$. Where, F_w , D_w , and T_w stands for fresh weight, dry weight and turgid weight, respectively.

Pooled analysis of variance (ANOVA) was used to quantify the genetic differences among the genotypes. The

multivariate analysis was performed through Window State Software using Mahalanobis' D^2 statistics (Mahalanobis, 1936). Treating D^2 as a generalized statistical distance, the criteria used by Toucher (Rao, 1952) was applied for determining the group constellation and clustering was done accordingly (Fig.1). The character-wise rank totals were used to calculate the per cent contribution of each character to the total divergence. Average inter- and intra-

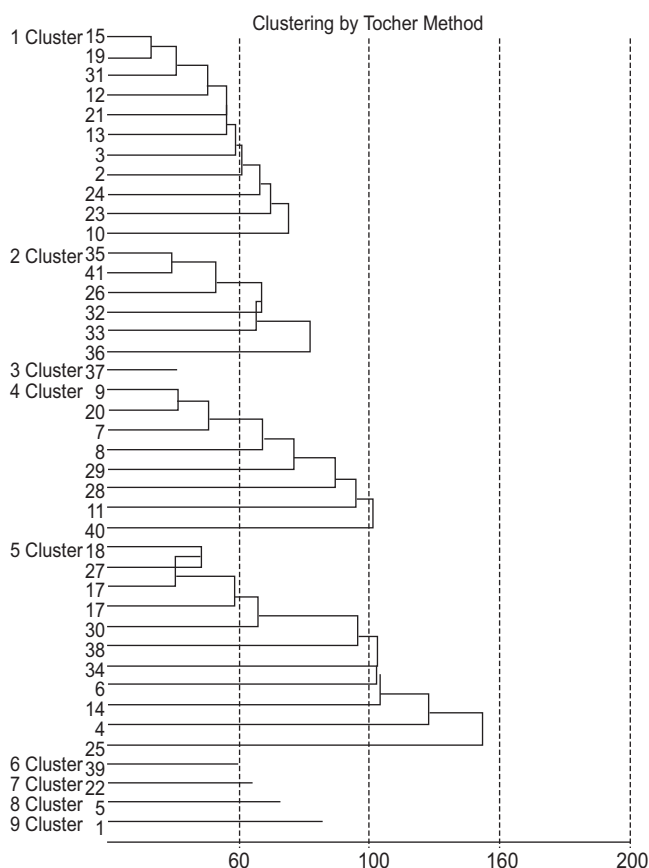


Fig. 1: Different clusters showing the position of inbreds (mentioned by serial number)

Table 2. Estimates of mean, range, mean square, phenotypic and genotypic coefficient of variation, heritability and genetic correlation with grain yield for different characters in maize

Characters	Mean	Range		Mean Square	PCV (%)	GCV (%)	h ² (broad sense)	rg with seed yield
		Min.	Max.					
Days to 50% pollen shed	59.02	52.00	66.17	82.938**	6.74	6.21	0.848	-0.440
Days to 50% silking	62.35	55.50	72.67	101.718**	7.07	6.51	0.848	-0.434
Anthesis silking interval	3.33	2.50	6.50	3.321**	29.55	20.60	0.486	-0.214
Days to maturity	93.87	83.67	108.67	160.385**	6.31	5.33	0.715	-0.160
Plant height (cm)	120.65	75.12	172.70	2878.41**	20.92	17.55	0.704	0.648
Ear height (cm)	53.43	32.30	83.93	856.202**	27.39	21.21	0.600	0.503
Number of leaves/ plant	10.93	9.50	13.03	3.823**	10.75	6.39	0.354	0.454
Number of barren plant/ plot	1.76	0.00	7.33	16.875**	83.61	60.89	0.640	-0.640
Number of ears/plant	1.10	0.62	1.33	0.121**	20.38	10.78	0.280	0.369
Ear length (cm)	11.43	7.70	15.85	22.00**	19.46	16.20	0.694	0.414
Ear girth (cm)	12.18	8.45	14.93	15.216**	14.43	12.79	0.786	0.800
Number of kernel rows	12.83	8.67	16.13	16.833**	14.76	12.69	0.739	0.622
Kernels/row	16.70	10.95	23.67	62.756**	22.66	18.55	0.670	0.372
100-kernel weight (g)	20.01	13.00	27.20	73.320**	17.78	17.41	0.959	0.688
Dry stover yield/ plant (g)	124.24	59.98	204.82	7836.555**	30.11	28.88	0.920	0.646
Biological yield/plant (g)	166.54	93.27	260.58	12114.983**	27.83	26.81	0.928	0.790
Canopy air temp. difference (°C)	0.00	-0.11	0.18	0.032	47.12	23.38	0.234	0.052
Relative water content (%)	75.00	59.00	88.06	339.794**	10.77	9.88	0.842	0.312
Proline content (mg/g fresh weight)	0.17	0.06	0.40	0.037**	49.91	45.35	0.826	-0.320
Grain yield/ plant (g)	42.31	19.87	69.65	893.056**	30.15	28.57	0.898	-----

*, ** Significant at P = 0.05 and 0.01, respectively

cluster distances were estimated as per the method given by Singh and Chaudhary (1985).

Results and Discussion

The analysis of variance revealed significant differences among genotypes for all the traits except canopy-air temperature difference (Table 2). Genotypes exhibited a wide range of variation for most of the traits except canopy-air temperature difference making possible the identification of lines with special characters. Similar results were also reported by Teixeira *et al.* (2002). The estimates of phenotypic coefficient of variation were greater than the genotypic coefficient of variation, indicating thereby the importance of environmental factors. High genotypic coefficient of variation (GCV) was found for number of barren plants, proline content, dry stover yield grain yield per plant, anthesis-silking interval (ASI), and ear height; moderate value of GCV were recorded for plant height, kernel rows per ear, 100-kernel weight and ear length.

The 41 genotypes were grouped into nine clusters (Fig. 1) using Tocher's method with variable number of entries (Table 3), suggesting adequate scope for selecting superior and diverse parents to be exploited for any breeding programme. Cluster I and V with 11 entries had the maximum number of genotypes followed by

Table 3 Distribution of 41 maize inbreds into different clusters

Cluster number	Number of inbreds	Name of inbred
I	11	CM139, CM144, LM9, LM6, CM132, CM121, CM142, CM122, CM207, CM116, Ib6118
II	6	Ib3010, M16-5, Y-500, Ib6135, Ib6140, PMS14
III	1	M-4-4
IV	8	CML228, CL02443, CM140, CM111, CM115, CM105, CM117 M-13-8
V	11	Y-501, Ib6105, M-7-7, Ib6144, LM12, LM10, CM138, CM137, CM135, CM125, CM213
VI	1	M-8-3
VII	1	CM143
VIII	1	LM11
IX	1	LM5

Table 4. Average intra-clusters (diagonal and bold) and inter-cluster distances (D^2 values) among nine clusters in maize

Clusters	I	II	III	IV	V	VI	VII	VIII	IX
I	7.89	16.75	17.15	9.55	11.34	16.15	9.22	10.70	12.50
II		8.70	10.16	18.08	12.76	10.63	19.26	21.57	16.72
III			0.00	17.34	14.12	7.93	20.25	22.15	15.70
IV				9.89	12.94	17.10	11.82	13.37	14.05
V					10.83	12.91	12.99	15.12	14.78
VI						0.00	19.48	19.43	17.67
VII							0.00	10.56	14.99
VIII								0.00	18.07
IX									0.00

cluster IV with eight genotypes representing different eco-geographical regions. Further, cluster II had six genotypes while rests of the clusters were solitary entry clusters. Similar results were reported by Zheng and Wang (1995), Singh *et al.* (1999) and Kumar and Singh (2002). The clustering pattern revealed that the genotype originated from different geographical regions had been grouped in cluster IV which indicated that there was no association between genetic diversity and geographical diversity. Similar results were reported by Sridhar *et al.* (2002). On the other hand, the genotypes that originated in one region had been distributed into different clusters, indicating that genotypes with same geographic origin could have undergone change for different characters under selection. This could be due to selection pressure, genetic drift and environment, which created greater diversity rather than genetic distance.

The intra- and inter-cluster distances (D^2 values) are presented in Table 4. Cluster V recorded maximum intra cluster distance followed by cluster I and cluster II, indicating existence of considerable genetic divergence among constituent genotypes. Hence parents within the cluster can be chosen for hybridization programme. The relative distance of each cluster from other clusters (inter-cluster distances) indicated greatest divergence between

cluster III and cluster VIII ($D=22.15$) followed by cluster III and VIII ($D=21.57$), II and VII ($D=20.25$), VI and VII ($D=16.09$), indicating greater diversity between genotypes belonging to respective pairs of clusters, whereas clusters III and VI were closest ($D=7.93$) with minimum genetic distance.

The cluster means of genotypes (Table 5) revealed considerable genetic differences between the groups. The cluster II registered the highest mean value for plant height (155.74) ear height (70.76), leaves per plant (11.85), kernels per row (18.13), dry stover yield (162.29) and biological yield per plant (218.95), whereas, the highest average cluster mean was reported for yield components like grain yield per plant in cluster VI (69.65) followed by cluster III (59.87); for 100-kernel weight in cluster III (26.65) and II (25.64); for number of kernel rows in cluster VI (16.13) and III (16.00); for kernels per row in cluster II (18.13) followed by cluster V (17.63); ears per plant in cluster VI (1.27) and for shorter plant height in cluster IX (108.90). The highest cluster means for character important to drought tolerance were observed in cluster VI for relative water content (88.06) and cluster VII for proline content (0.26). The characters whose low value is desirable for drought tolerance, the lowest mean values were obtained in cluster V (92.20) and II (92.42)

Table 5. Cluster means and percent contribution of characters towards divergence in maize

Clusters Characters	I	II	III	IV	V	VI	VII	VIII	IX	Number of times ranked 1st	Contribution %
Days to 50% pollen shed	60.65	56.00	59.83	59.67	57.24	58.17	61.00	64.67	66.17	58	7.07
Days to 50% silking	64.15	59.22	62.67	62.58	60.58	61.17	64.00	68.00	72.67	1	0.12
Anthesis silking interval	3.50	3.22	2.83	2.92	3.33	3.00	3.00	3.33	6.50	37	4.51
Days to maturity	95.26	92.42	96.00	92.73	92.20	96.33	95.00	94.17	108.67	1	0.12
Plant height (cm)	110.30	155.74	131.00	109.76	120.28	129.25	109.13	119.43	108.90	1	0.12
Ear height (cm)	49.76	70.76	56.60	49.68	51.45	47.73	53.43	49.27	48.40	1	0.14
Number of leaves/ plant	10.61	11.85	10.72	10.51	11.04	10.38	11.60	11.33	10.77	3	0.40
Number of barren plant/ plot	2.67	0.72	0.00	2.15	1.03	0.00	2.67	0.17	7.33	23	2.85
Number of ears/plant	1.12	1.16	1.00	1.03	1.15	1.27	1.13	1.08	0.62	10	1.21
Ear length (cm)	10.92	12.59	10.08	10.80	12.19	9.73	9.33	13.62	9.75	13	1.59
Ear girth (cm)	11.14	13.92	14.33	10.86	12.83	14.93	10.75	13.12	12.00	14	1.71
Number of kernel rows	12.13	13.88	16.00	11.74	13.09	16.13	14.37	14.67	10.00	6	0.70
Kernels/row	16.33	18.13	15.45	15.83	17.63	16.73	14.83	16.33	12.33	12	1.51
100-kernel weight (g)	17.60	25.64	26.65	18.29	20.61	22.80	15.98	13.00	21.43	252	30.85
Grain yield/plant (g)	34.14	56.69	59.87	33.11	47.67	69.65	31.95	37.72	30.40	67	8.17
Dry stover yield/ plant (g)	105.99	162.29	116.40	103.94	142.35	150.12	114.87	101.38	73.83	127	15.61
Biological yield/plant (g)	140.13	218.98	176.27	137.05	190.02	216.77	146.82	139.10	104.23	10	0.15
Canopy air temp. difference (°C)	0.00	0.00	0.04	-0.02	0.02	-0.03	-0.06	0.04	0.09	0	0.00
Relative water content (%)	71.84	75.39	84.92	81.73	73.67	88.06	59.00	68.54	67.54	96	11.71
Proline content (mg/g fresh weight)	0.16	0.10	0.16	0.24	0.17	0.08	0.26	0.10	0.16	94	11.46

for early maturity, cluster III (2.83) for ASI and cluster III (0.00) and VI (0.00) for barren plants. The clusters contributing maximum to D^2 values are to be given greater emphasis for deciding the clusters for the purpose of further selection and hybridization. The characters contributing towards genetic divergence show that maximum genetic divergence was observed for 100-kernel weight (30.85%) followed dry stover yield (15.61%), relative water content (11.71%), proline content (11.46%) and grain yield per plant (8.17). Hence, 100-kernel weight, dry stover yield relative water content, proline content and grain yield were considered to be important traits. The least and negligible contribution towards divergence was observed for canopy air temperature difference (0.00%). Similar results were earlier reported by Kumar and Singh (2002), Teixeira *et al.* (2002) and Singh *et al.* (2005).

The data on inter-cluster distances and *per se* performance of genotypes may be used to select genetically diverse and agronomically superior genotypes. On the basis of inter cluster distances, the eight genotypes, viz., M-4-4, Ib3010, M16-5, Y-500, Ib6135, Ib6140, PMS14 and LM11 listed in cluster II, III and Cluster VIII could be used to obtain the heterotic combinations in hybridization programme. Intercrossing of divergent groups would lead to greater opportunity for crossing over, which may release hidden variability by breaking linkage (Thoday, 1960). Progenies derived from such diverse crosses are expected to show wide spectrum of genetic variability. Hence, these genotypes might be used in single as well as multiple crossing programmes for development of hybrids.

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Genetic Diversity in Rice (*Oryza sativa* L.) Genotypes Resistant and Susceptible to the Asian Rice Gall Midge *Orseolia oryzae* (Wood-Mason)

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Genetic diversity among nine gall midge [*Orseolia oryzae* (Wood-Mason)] resistant and three susceptible rice (*Oryza sativa* L.) genotypes, comprising of cultivars, breeding lines and landraces, was studied in terms of polymorphism using 358 SSR primers representing the whole genome. Of the 154 polymorphic primers, 82 were found to be highly polymorphic and informative. Number of alleles amplified by these primers ranged from two to seven. Highest polymorphism (40.7%) was noted with primers from chromosome 3. The average of polymorphism information content (PIC) was 0.589. Cluster analysis based on the allelic diversity clearly demarcated the cultivars, landraces and breeding lines into different groups. The genotypes did not distinguish into gall midge resistant and susceptible groups. Two of the landraces, Aganni and Jhitpiti, showed least diversity despite diverse geographical sources of their collection. One of the breeding lines, RP2068-18-3-5, displayed distinctness from all the other genotypes tested. Diversity among the genotypes tested is more evident with respect to directed breeding efforts through human intervention rather than natural evolution of gall midge resistance.

Key Words: SSR polymorphism, Cultivars, Breeding lines, Landraces, Resistance gene evolution

Introduction

The Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) (Diptera: Cecidomyiidae) is one of the important insect pests of rice. Breeding and cultivation of resistant rice varieties has been an important strategy to manage this pest. Field and greenhouse evaluation of over 50,000 germplasm accessions of rice has resulted in identification of over 300 primary sources of resistance (Bentur *et al.*, 2003). Utilizing some of these sources, over 60 gall midge resistant rice varieties have been developed and released for commercial cultivation in India since 1972. Genetic analysis of some of these sources led to the characterization of nine dominant and one recessive resistance genes (Kumar *et al.*, 2005). Several of the resistant genotypes carry the same resistance gene as revealed by the classical genetic studies (Kumar *et al.*, 1998a) or through the use of flanking Simple Sequence Repeat (SSR) markers (Himabindu *et al.*, 2007). While the diversity at the gall midge resistance locus in selected rice genotypes has been well studied (Kumar *et al.*, 2005, Biradar *et al.*, 2004, Himabindu *et al.*, 2007), genome-wide diversity of genotypes carrying either the same or different resistance genes has not been studied. Such studies may reveal convergent evolution of same or similar resistance gene(s) in geographically distinct landraces or divergent evolution of genotypes carrying the same gene.

Molecular markers are being extensively used in the study of genetic diversity of crop plants to address questions

of phylogeny, evolution and adaptation through domestication (Wang *et al.* 1992; Joshi *et al.*, 2000; Bautista *et al.*, 2001; Ren *et al.*, 2003). SSRs are useful markers for genome analysis, because of the significant level of allelic diversity that they reveal (Ishii *et al.*, 2000). The SSR markers are supposed to be particularly suitable for evaluating genetic diversity among closely related plant accessions or genotypes, such as different rice cultivars (Ren *et al.*, 2003). These markers have been used in several studies like genetic diversity in different crops (Ellwood *et al.*, 2006), germplasm analysis (Yong *et al.*, 2002), genetic relationships (Zhang *et al.*, 2003), phylogenetic studies (Provan *et al.*, 2001) and others. In rice over 25,000 SSR markers are available (Mc Couch *et al.*, 2002) which make them ready tool for genetic diversity analysis.

In the present study, we report genetic diversity analysis based on genome wide polymorphism in SSR loci in respect of a set of nine gall midge resistant and three gall midge susceptible genotypes representing cultivars, breeding lines and landraces. Our results showed diversity between cultivars and landraces but not dependent on gall midge resistance.

Materials and Methods

Nine gall midge resistant rice genotypes used in this study included three cultivars: Kavaya, Phalgun, Abhaya; two breeding lines: RP2068-18-3-5, CR57-MR1523 and four landraces: Aganni, ARC15831, INRC3021 and Jhitpiti. Three gall midge susceptible cultivars used were TN1,

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B95-1 (Improved Samba Mahsuri) and IR64. Details of these genotypes along with their reaction against different gall midge biotypes are given in Table 1.

The selected genotypes were screened in the greenhouse for the reaction against gall midge biotypes (GMB) 1, 4 and 4M, following the standardized screening procedure described elsewhere (Vijaya Lakshmi *et al.*, 2006). Total DNA was extracted from leaf tissue of selected genotypes by the cetyl tri-methyl ammonium bromide (CTAB) method (Rajendrakumar *et al.*, 2007). Conditions for PCR set up and electrophoresis were similar to those described by Himabindu *et al.* (2007).

A set of 358 SSR primer pairs (markers) covering the entire rice genome was used for PCR amplification (Mc Couch *et al.*, 2002). The size of the amplified fragments was calculated using AlphaEase software (Alpha Innotech, USA) with I/HindIII+EcoRI double digest and 100-bp ladder (MBI Fermentas, Lithuania) as size reference standard. These values are used to construct a dendrogram using NTSYSpc software (version 2.0).

Results

Of the 358 SSR markers tested, 154 showed polymorphism among the test genotypes. Of these, 82 were most informative in being highly polymorphic. A total of

1,161 alleles were amplified with these markers. Number of alleles per marker ranged from two to seven, with an average of 3.24. Among the markers tested, per cent polymorphism varied from 4.6 on chromosome 6 to 40.7 on chromosome 3. Map distance covered by the SSR markers ranged from 0.003 Mb on chromosome 8 (between the markers RM23246 and RM23248) to 11.3 Mb on chromosome 6 (between the markers RM111 and RM20060), as per the reported location of the markers on rice genome (Mc Couch *et al.*, 2002). The average of polymorphism information content (PIC) was 0.589.

Fourteen most informative SSR markers dispersed across the 12 chromosomes were identified based on polymorphism percentage with the resistant and susceptible genotypes. A positive correlation was found between number of repeats within the microsatellite loci, and the extent of polymorphism between the genotypes ($R^2 = 0.28$). Six of the 14 most informative SSR markers were with AT repeat motif. The breeding line RP2068-18-3-5 showed maximum polymorphism (23.5-25.7%) with all the three susceptible genotypes.

Genetic diversity among the test genotypes was analyzed using the polymorphism data from the 82 highly polymorphic and informative SSR markers. A dendrogram was constructed based on the similarity coefficient among

Table 1. Rice genotypes selected for parental polymorphism study

S. No.	Genotype	Parentage/Native state	Type of genotype	Grain Type [†]	Resistance Gene	Reaction pattern [§]
1	Kavya	WGL 27120/ WGL 17672// Mahsuri/ Surekha	Cultivar	LS	<i>Gm1</i>	R-S-S
2	Phalguna	IR 8/ Siam 29	Cultivar	LS	<i>Gm2</i>	R-S-S
3	RP2068-18-3-5	Swarnadhan/ Velluthacheera	Breeding line	LB	<i>gm3</i>	R-R-R
4	Abhaya	CR 157-392/ OR 57-21	Cultivar	MS	<i>Gm4</i>	R-R-R
5	Jhitpiti	Chhattisgarh	Landrace	LB	<i>Gm8</i>	R-R-R
6	CR57-MR1523	IR8/ Ptb 21	Breeding line	LS	<i>Gm11 (t)</i>	R-R-S
7	Aganni	Kerala	Landrace	LB	<i>Gm?</i>	R-R-R
8	ARC15831	Assam	Landrace	LS	<i>Gm?</i>	R-R-R
9	INRC3021 (Ajan)	Chhattisgarh	Landrace	LS	<i>Gm?</i>	R-R-R
10	TN1	Chow-wu-gen/ Tsai-vuvan-chung	Cultivar	MB	None	S-S-S
11	BPT5204 (Improved Samba Mahsuri)	BPT 5204*4/ SS1113	Cultivar	MS	None	S-S-S
12	IR64	IR5657-33-2-1/ IR 2061-465-1-5-5	Cultivar	LS	None	S-S-S

[†] LB- Long bold, LS- Long slender, MB- Medium bold, MS- Medium slender.

[§] Based on the reaction observed against the gall midge biotypes 1, 4 and 4M; R-Resistant, S-Susceptible (modified after Vijaya Lakshmi *et al.*, 2006).

the genotypes (Fig. 1). This dendrogram grouped the test genotypes into three distinct groups at a similarity coefficient level of 0.58. The first group included all the six cultivars tested including three gall midge resistant cultivars viz., Phalgun, Abhaya and Kavya and three susceptible cultivars IR64, B95-1 and TN1. One of the breeding lines CR57-MR1523 was also a member of this group. The second group included all the four landraces - ARC15831, Jhitpiti, Aganni and INRC3021. Jhitpiti and Aganni showed over 87% similarity and were not separated in the dendrogram. The second group differed from the first by recording 0.61 similarity coefficient. The third group had a lone member, the breeding line RP2068-18-3-5 which stood at the farthest of similarity coefficient level of 0.58.

Discussion

The genotypes tested in the present study represented three levels of human intervention. The landraces were least intervened. Breeding lines were the product of targeted hybridization for developing improved lines but were not carried to the logical end for want of desirable agronomic traits and performance. Cultivars represented the final product of plant breeders and were released as varieties for commercial cultivation. Among the cultivars

included in this study, IR64 and BPT5204 are being cultivated on the largest area of four and three million hectares in India (Singh, 2006). B95-1 is the improved form of BPT5204 through marker assisted pyramiding of bacterial leaf blight resistance genes *xa21*, *xa13* and *xa5*. The intensive selection pressure that all the cultivars Kavya, Phalgun and Abhaya might have gone through, was evident in the least range of genetic diversity among them (CL > 0.67) and being clustered in group I. Greater divergence of Kavya within the group could be due to the divergent parents to begin with. The breeding line CR57-MR1523 fell within this group with lesser divergence than the cultivar Kavya, suggesting the potential of this line to have become a cultivar. Utilizing this pre-breeding line developed at the Central Rice Research Institute at Cuttack, India, the Directorate of Rice Research developed the cultivar – Suraksha – with multiple pest resistance and released for commercial cultivation in 1988 (Shobharani *et al.*, 2008). Though all the six cultivars tested were in the first group, gall midge susceptible cultivars did not make a separate sub-group as against the resistant cultivars. Thus there was no significant divergence among the cultivars based on gall midge resistance or susceptibility.

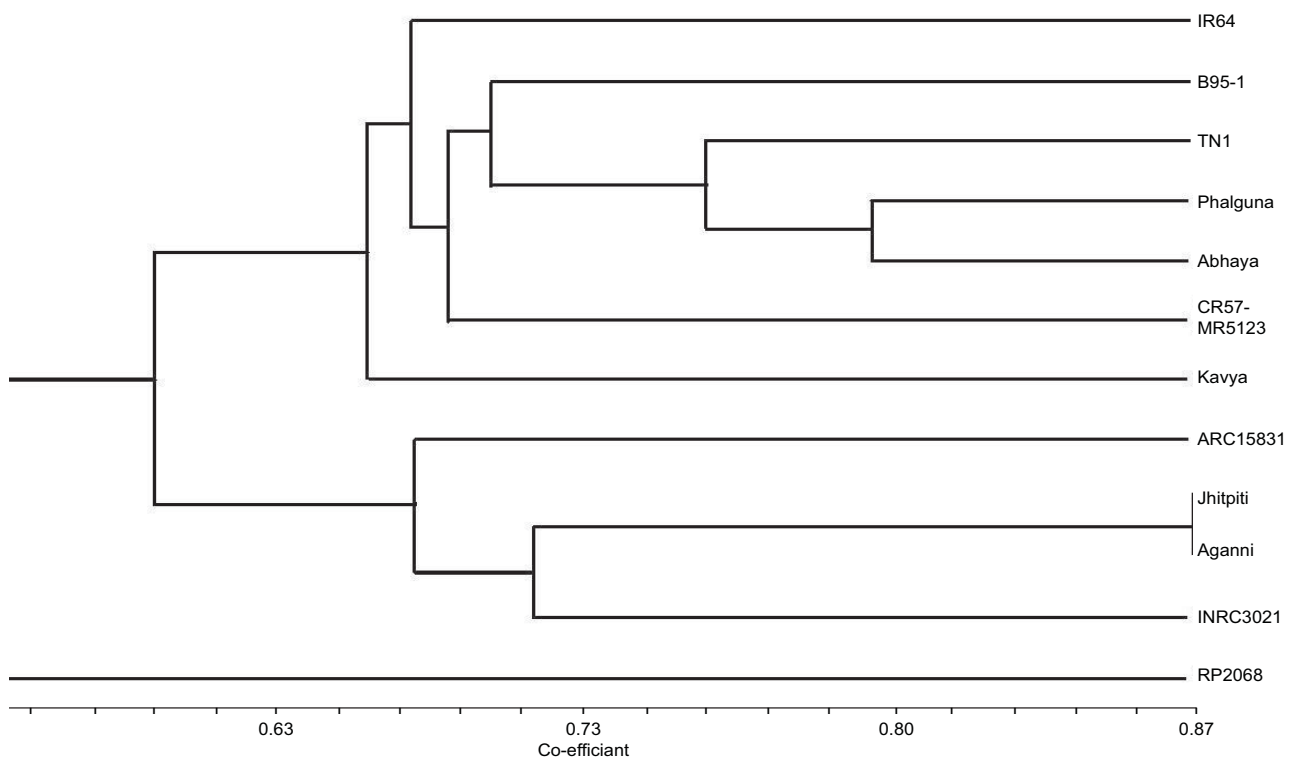


Fig. 1: Dendrogram resulting from NTSYS cluster analysis of the 12 rice genotypes, based on polymorphism data derived from 82 highly polymorphic SSR markers

Among the landraces in the second group, Jhitpiti and Aganni did not show any discernable divergence at all. Geographically, Jhitpiti has been collected from the state of Chhattisgarh while Aganni is a landrace from the southern state of Kerala. While Jhitpiti has a characterized gall midge resistance gene *Gm8* located on the chromosome 8, Aganni has yet uncharacterized resistance gene, probably located also on the chromosome 8 (Sama *et al.*, unpublished). Such a convergence of two landraces, geographically distinct, possessing gall midge resistance is unique. ARC 15831 represented one of the accessions of Assam Rice Collections made during early 1960s which has contributed to a large pool of gall midge resistant donors (Bentur *et al.*, 2003). INRC 3021, the landrace – Ajan – also collected within the state of Chhattisgarh, like Jhitpiti, showed greater divergence from the latter when compared to Aganni with a similarity coefficient of 0.72.

The third group with the lone member, RP2068-18-3-5, recorded highest divergence. This is a breeding line derived from the cross Swarnadhan, a popular rainfed lowland cultivar, and Velluthacheera, a multiple pest resistant landrace from the southern state of Tamil Nadu. This breeding line is also unique in possessing the only recessive gall midge resistance gene, *gm3* (Kumar *et al.*, 1998b), with the expression of hypersensitive reaction (HR).

Our study using SSR markers showed three distinct clusters of genetic diversity between cultivars, landraces and a breeding line independent of gall midge resistance. Limited diversity was seen among cultivars. Thus large number of mapped SSR markers can be efficiently used for evaluation of genetic diversity and estimation of genetic relationships between *O. sativa* genotypes of different origin.

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Inter-trait Association and Genetic Variability Assessment in Snapmelon (*Cucumis melo* var. *momordica*)

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Genetic variability, heritability (h^2), genetic advance, character association and path analysis were carried out in 74 accessions of snapmelon (*Cucumis melo* var. *momordica*) for 12 quantitative characters. High phenotypic (PCV) and genotypic (GCV) coefficient of variation was observed for fruit weight and yield per plant. High heritability along with high expected genetic advance was recorded for polar and equatorial circumference of fruit. Yield had positive and significant correlation with fruit weight, polar and equatorial circumference of fruit at both phenotypic and genotypic level and with days to first female flower anthesis at genotypic level. Fruit weight, polar and equatorial circumference of fruit had positive correlation coefficient among themselves. Higher and positive direct effect on yield was exerted by number of fruits per plant followed by fruit weight, polar and equatorial circumference of fruit, days to first male flower anthesis and node at which first female flower appears. Besides, direct selection for fruit weight, indirect selection through polar and equatorial circumference of fruit could be considered for further improvement of yield.

Key Words: Snapmelon, Variability, Heritability, Genetic advance, Correlation, Path analysis

Introduction

Snapmelon (*Cucumis melo* var. *momordica*), belongs to family Cucurbitaceae, is used as vegetable in a variety of ways. Snapmelon is rich in quality and its juice is gaining popularity as squash. Evaluation of the variability is prerequisite for a quality improvement programme. Germplasm from heterogeneous environment are genetically diverse and could provide scope for screening better genotypes with specific traits. Knowledge about the nature and magnitude of variation provide a rational choice of character(s) on which selection can be exercised. The observed variability is a combined estimate of genetic and environmental factors, of which only former one is heritable. However, the estimate of heritability alone does not provide an idea about the expected gain in next generation, therefore it is generally considered in conjunction with genetic advance. Correlation and path analysis establish the extent of association between yield and its components and also bring out relative importance of their direct and indirect effects. This gives a clear understanding of their association with yield. In the present study, an assessment was made about the performance of various economic traits and the extent of variability, heritability, expected genetic advance and interrelationship of yield components in snapmelon was measured.

Materials and Methods

Seventy-four accessions of snapmelon were grown in a completely randomized block design (CRBD) with three

replications with row-to-row distance of 3 m and plant-to-plant distance of 60 cm during rainy season of 2007. Eight plants were maintained in each plot. Standard cultural practices were followed and recommended dose of fertilizers were given to raise a good crop. The observations were recorded on five random plants for each treatment in each replication for days to first male flower anthesis, days to first female flower anthesis, node at which first male flower appears, node at which first female flower appears, primary branches per plant, vine length (cm), polar and equatorial circumference of fruit (cm), fruit weight (kg), number of fruits per plant, total soluble solids (TSS) and yield per plant (kg). The data thus obtained was subjected to various statistical analysis for estimating the genotypic and phenotypic coefficient of variation (Burton, 1952), heritability (Hanson *et al.*, 1956), and genetic advance (Johnson *et al.*, 1955). Phenotypic and genotypic correlation coefficients were calculated following the procedure of A1-Jibouri *et al.* (1958) and path analysis was carried out according to the method of Dewey and Lu (1959).

Results and Discussion

Significant differences were observed among the genotypes for different characters under study (Table 1). A wide range of variability was recorded for fruit weight (0.1-3.9), yield per plant (0.4-9.4), polar circumference of fruit (12.8-96.4), equatorial circumference of fruit (10.2-54.4) and node at which first female flower appears (3.0-17.0). The higher phenotypic coefficient of variation (PCV) than those of genotypic coefficient of variation

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Table 1. Range, mean, variability, heritability, genetic advance and genetic advance in per cent of mean

Characters	Range	Mean	Variability		Heritability (h ²) advance	Genetic	Genetic advance in per cent of mean
			PCV	GCV			
Days to 1 st male flower anthesis	42.0-52.0	46.5	8.3	3.5	17.4	1.4	3.0
Days to 1 st female flower anthesis	43.3-57.0	51.7	9.0	0.79	0.8	0.07	0.1
Node at which 1 st male flower appears	2.3-4.3	3.3	13.1	10.4	62.7	0.6	16.9
Node at which 1 st female flower appears	3.0-17.0	8.4	32.5	31.5	94.0	5.3	62.9
Primary branches/ plant	4.0-12.0	6.2	22.0	20.3	85.6	2.4	38.7
Vine length (m)	2.2-5.9	3.6	19.9	17.0	77.7	1.1	30.8
Polar circumference of fruit (cm)	12.8-96.4	44.0	37.9	36.8	94.1	32.3	73.5
Equatorial circumference of fruit (cm)	10.2-54.4	27.5	29.5	28.0	90.3	15.1	54.7
Fruit weight (kg)	0.1-3.9	0.9	76.4	75.5	97.7	1.3	144.1
Number of fruit/plant	2.0-13.4	4.9	56.8	55.7	95.9	5.5	112.3
TSS (%)	2.2-5.0	3.8	19.4	17.7	83.0	1.3	33.2
Yield/plant (kg)	0.4-9.4	3.2	58.9	57.3	94.6	3.7	114.7

(GCV) indicated the predominant role of environment on the expression of the traits. The maximum phenotypic and genotypic coefficient of variation was observed for fruit weight followed by yield per plant, number of fruits per plant and polar circumference of fruit, while the lowest PCV was observed for days to first male flower anthesis, followed by days to first female flower anthesis and node at which first male flower appears. The lowest value of genotypic coefficient of variation was observed for days to first female flower anthesis, followed by days to first male flower anthesis. These results are in agreement to the findings of Sahni *et al.*, (1987) in ridge gourd, Dahiya *et al.*, (1989) in roundmelon, Singh *et al.*, (1989) in muskmelon, Prasad and Singh (1992) in cucumber and Pandey *et al.*, (2003) in snapmelon. The variation was also observed for plant growth characteristics viz., ridges on fruit surface, fruit cracking pattern and skin peeling, fruit shape, fruit skin texture, predominant primary and secondary color of fruits, design produced by secondary skin colour and flesh colour (Table 2).

High estimate of heritability in broad sense was obtained for all the characters except days to first female and first male flower anthesis. According to Panse (1957), the magnitude of heritable value is the most important aspect of genetic constitution of breeding material, which has close bearing on the response to selection. The high value for heritability was obtained for fruit weight (97.7), number of fruits per plant (95.9), yield per plant (94.6), polar circumference of fruit (94.1), node at which first female flower appears (94.0) and equatorial circumference of fruit (90.3). These findings indicate that there is a scope for improvement in these characters through direct selection. In the present study, fruit weight showed the

maximum genetic gain (144.1) followed by yield per plant (114.7) and number of fruits per plant (112.3). Earlier, in snapmelon, Pandey *et al.*, (2003) reported high genetic advance for yield per plant.

Heritability along with genetic gain is more useful criteria in predicting the resultant effect for selecting the best individual (Johnson *et al.*, 1955). Burton (1952) suggested that characters with high heritability coupled with high genetic advance would show better response to selection than those with high heritability and low genetic advance. In present study, high heritability along with high genetic gain was recorded for polar and equatorial circumference of fruit. These results are in accordance with the findings of Pandey *et al.*, (2002) in pumpkin.

Table 2. Frequency of descriptor states for qualitative descriptors in snapmelon

Trait	Descriptor state (number of genotype)
Ridges on fruit surface	Present (20), Absent (54)
Fruit cracking pattern and skin peeling	No cracking (30), Random cracking (6), Longitudinal cracking (25), Random skin peeling (9), Blossom end cracking (4)
Fruit shape	Cylindrical (12), Oval (29), Elongate (18), Globular (4), Round (5), Pyriform (3), Curved (1), Oblong (2)
Fruit skin texture	Plain (73), Netted (1)
Fruit skin predominant primary colour	Green (40), Dark green (20), Light green (14)
Fruit skin predominant secondary colour	Yellow (35), Orange (7), Brown red (8), Brown cream (1), Light green (9), White (6), Cream (6), Brown (2)
Design produced by secondary skin colour	No design (42), Stripped (19), Streaked (4), Spotted (7), Speckled (2)
Flesh colour	Orange (20), White (12), Cream (42)

The present results indicate that phenotypic selection could bring about improvement in these characters. It is suggested that variation observed for different characters in snapmelon genotypes may be due to the high additive gene effect. High heritability and low genetic gain were observed for node at which first male flower appears and TSS. This reflects that high heritability is not always associated with genetic advance (Swarup and Chaugale, 1962). Hence, selection on the basis of these characters will be less effective since they are controlled by non-additive genes.

Genotypic correlation coefficient was higher than their corresponding phenotypic correlation coefficient for most of the characters except node at which first male flower appears, number of primary branches per plant and TSS (Table 3). It was observed that yield has positive and significant correlation with fruit weight, polar and equatorial circumference of fruit at both genotypic and phenotypic level and with days to first female flower

anthesis at genotypic level. It has been reported that fruit weight, polar and equatorial circumference of fruit had positive association among themselves (Vijay, 1987 and Pandey *et al.*, 2003). Higher and positive direct effect (at the genotypic level) on yield was exerted by number of fruits per plant followed by fruit weight, polar and equatorial circumference of fruit, days to male flower anthesis and node at which first female flower appears (Table 4). All the above mentioned characters also showed positive correlation with yield. This indicates that direct selection based on number of fruits per plant, fruit weight and polar and equatorial circumference of fruit could result in an appreciable improvement of total yield. However, vine length, number of primary branches, TSS, node at which first male flower appears and days to first female flower anthesis exerted negative direct effect on yield. Similar results have also been reported in snapmelon (Pandey *et al.*, 2003) and muskmelon (Pandey *et al.*, 2005). From the results of this investigation, it is concluded

Table 3. Estimates of genotypic and phenotypic correlation coefficients of yield and its contributing traits

Traits	Days to 1 st female flower anthesis appears	Node at which 1 st male flower appears	Node at which 1 st female flower /plant	Number of primary branches	Vine length (m)	Polar circumference of fruit (cm)	Equatorial circumference of fruit (cm)	Fruit weight (kg)	Number of fruits/plant	TSS (%)	Yield/plant (kg)
	2	3	4	5	6	7	8	9	10	11	12
1 rg	0.824**	-0.099	0.025	0.281*	0.003	-0.001	0.134	-0.069	0.232*	-0.025	0.206
rp	0.784**	0.032	0.023	0.072	0.038	-0.060	0.041	-0.053	0.098	-0.099	0.057
2 rg		-0.482	0.631**	0.543**	0.015	0.962**	1.291**	0.509**	0.295**	-0.440	1.318**
rp		-0.011	0.051	-0.001	0.004	0.006	0.066	0.008	0.012	-0.110	0.058
3 rg			0.094	-0.182	0.045	0.013	0.122	0.154	-0.187	0.012	-0.005
rp			0.040	-0.133	0.061	0.004	0.071	0.117	-0.139	-0.006	0.012
4 rg				-0.131	-0.021	-0.116	-0.170	-0.119	0.021	-0.008	-0.100
rp				-0.136	-0.012	-0.113	-0.153	-0.110	0.021	-0.011	-0.100
5 rg					-0.046	-0.196	-0.170	-0.033	0.370**	0.000	0.017
rp					-0.044	-0.177	-0.151	-0.032	0.346**	-0.013	0.031
6 rg						0.404**	0.414**	0.311**	-0.290	-0.204	0.195
rp						0.301**	0.320**	0.253*	-0.241	-0.131	0.157
7 rg							0.663**	0.817**	-0.535	-0.277	0.708**
rp							0.644**	0.810**	-0.510	-0.246	0.692**
8 rg								0.804**	-0.576	-0.191	0.651**
rp								0.778**	-0.538	-0.201	0.627**
9 rg									-0.508	-0.129	0.784**
rp									-0.491	-0.122	0.776**
10 rg										0.265*	-0.064
rp										0.232*	-0.042
11 rg											-0.122
rp											-0.128

* Significant at 5% probability, ** Significant at 1% probability level

Table 4. Direct (diagonal) and indirect effect of different traits on yield (genotypic)

Traits	Days to 1 st male flower anthesis	Days to 1 st female flower anthesis	Node at which 1 st male flower appears	Node at which 1 st female flower /plant	Number of primary branches	Vine length (m)	Polar circumference of fruit (cm)	Equatorial circumference of fruit (cm)	Fruit weight (kg)	Number of fruits/plant	TSS (%)	Yield/plant (kg)
	1	2	3	4	5	6	7	8	9	10	11	12
1	0.090	-0.006	0.003	0.001	-0.024	0.000	0.000	0.042	-0.040	0.139	0.002	0.206
2	0.074	-0.007	0.016	0.027	-0.047	-0.002	0.354	0.401	0.294	0.177	0.029	1.318**
3	-0.009	0.003	-0.034	0.004	0.016	-0.005	0.005	0.038	0.089	-0.112	-0.001	-0.005
4	0.002	-0.005	-0.003	0.043	0.011	0.002	-0.043	-0.053	-0.069	0.013	0.001	-0.100
5	0.025	-0.004	0.006	-0.006	-0.087	0.005	-0.072	-0.053	-0.019	0.222	0.000	0.017
6	0.000	0.000	-0.002	-0.001	0.004	-0.103	0.149	0.129	0.180	-0.174	0.014	0.195
7	0.000	-0.007	0.000	-0.005	0.017	-0.042	0.368	0.206	0.473	-0.321	0.018	0.708**
8	0.012	-0.009	-0.004	-0.007	0.015	-0.043	0.244	0.311	0.465	-0.345	0.013	0.651**
9	-0.006	-0.004	-0.005	-0.005	0.003	-0.032	0.301	0.250	0.579	-0.305	0.009	0.784**
10	0.021	-0.002	0.006	0.001	-0.032	0.030	-0.197	-0.179	-0.294	0.599	-0.018	-0.064
11	-0.002	0.003	0.000	0.000	0.000	0.021	-0.102	0.060	-0.074	0.159	-0.066	-0.122

** Significant at 1% probability level, Residual effect-0.131

that besides direct selection for fruit weight, indirect selection through polar and equatorial circumference of fruit should be considered for further improvement of yield in snapmelon.

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Mutations: In Crop Evolution, Improvement and Reverse Genetics

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Mutation has been used as efficient tool for plant improvement and is also considered as one of the important factors involved in crop evolution. Spontaneous mutations known to occur in nature have led to development of new cultivars. During the last few decades it has become an effective tool in reverse genetics. The paper reviews the various roles of mutation in crop evolution, plant improvement and reverse genetics.

Key Words: Mutations, Macro evolution, Reverse genetics

Most efficient tool available to plant breeders in their quest to develop improved cultivars and a scientific cause of crop evolution is mutation. A mutation is a change in the structure as well as function of a gene. In most cases, it is deleterious as it means that the gene doesn't produce what it should. However, mutations can result in valuable new traits. Spontaneous mutations occur in nature at a relatively continuous and frequent rate. In almost all the crops species, large numbers of spontaneous mutations have been recorded and used either directly as new cultivars or breeding line in breeding programs. In all vegetatively propagated crops, mutations are being used successfully for developing new cultivars (e.g. changes in fruit color or time of fruit maturity, etc). Recent decades have witnessed intensive work on the induction of mutations by using irradiation, chemicals and other mutagenic agents. The frequency of induced mutations almost doubles those occurring naturally and they have been looked on as a powerful tool for the development of new cultivars. However, available mutagens cause not only changes in genes but also chromosomal aberrations, many of which are deleterious in their effect on the trait and on the entire organism. Consequently, there have been a limited number of induced mutations directly usable as new cultivars. Since mutation particularly spontaneous mutation is an ultimate source of variation and speciation in entire living organisms that ultimately resulted in natural evolution of cultivated species from their wild sources. In the mid of 19th Century induced mutation became a most effective tool for crop improvement and in the beginning of 21st century mutations has become an efficient tool in the reverse genetics.

Mutations in Crop Evolution

Biological evolution is the natural process that explains the common relationship of all life on earth now and in

the past. Evolution is a result of the combined changes that occur in a **population** of **organisms** over successive generations. All living organisms can **inherit** these changes because they have genes, which are made up of molecules called **DNA**. Changes in these molecules, called (**mutations**), can become a new feature in the offspring of a living organism. These new features called **traits** are almost always minor. Because none of the organisms have exactly the same traits, they will live and reproduce differently, some more effectively than others. Scientists call this process **natural selection**. Over the time, the favorable trait will become common in the descendents of the creatures. Evolution is a continuous process, resulting from accumulation of minor mutations most of which exert only small effects.

Mutation
Gene Flow + **3.8 billion years** = **Macroevolution**
Genetic Drift
Natural Selection

Though inconsistent in his views, Darwin also considered that the slight and fluctuating variations are of more importance. Baur (1924) repeatedly emphasized greater importance of small mutations in evolution. Most of the evolutionists like Stebbins (1950) and Dobzhansky (1951) recognized the small steps in form of minor mutations as the most significant steps of evolution and speciation. Crop plant domestication began approximately 10,000 years ago at the dawn of agriculture (Harlan, 1992). During the domestication process, early agriculturists consciously or unconsciously selected among wild germplasm for material that was better adapted to human use and cultivation. Since the transition from wild species to domesticate, crop plants have continued to change due to selection exerted by ancient and modern plant breeding and cultivation practices. These changes

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that occurred subsequent to the initial domestication event(s) are known as crop evolution. As a result of both crop domestication and evolution, today's domesticated species are differentiated from their wild ancestors by an assortment of morphological and physiological characteristics. This is true despite the often large differences in gross morphology between crop and their progenitors because artificial (human) selection is likely to have acted primarily on the small sites of genes controlling morphogenetic traits of interest to humankind, leaving the vast majority of genome to evolve at much slower pace.

Mutations in Crop Improvement

The selection of better natural genetic variation and creation of new such variations followed by selection of desirable ones are the major activities in plant breeding. New genetic variability can be created through conventional and non-conventional breeding methods, namely, introduction, selection, hybridization, polyploidy, mutation breeding, etc. According to Brock (1971) induced genetic variation represents a more efficient source of genetic variability in the gene pools conserved by nature. Since genetic variability is a prerequisite for any successful breeding programme, the creation and management of genetic variability becomes central base for improvement of any crop species. Selection and hybridization can be used as conventional methods for the improvement of qualitative as well as quantitative traits, but in a highly self-pollinated crop, these methods with a limited genetic variability do not prove so effective. Spontaneous mutations occur in natural population and give rise to important natural variations. Part of the possible variations arising in a plant population is due to spontaneous mutations, which mostly arise at a very low frequency of 10^{-5} – 10^{-8} per locus, and for various reasons often remains undetected and unexploited. Therefore, spontaneous mutation can not be expected to serve the cause of crop improvement effectively. In this context, it is quite desirable to select and apply induced mutagenesis, which is recognized as a quick and successful method in creating genetic variability and bringing about desirable improvement.

A very good example of spontaneous and induced mutations is the one which resulted in green revolution in Asia and Africa. There are many genes associated with a semidwarf growth habit in wheat (Ellis *et al.*, 2005). They are known as *reduced height* (*Rht*) genes, and many of them are dominant or semidominant, indicating that

they actively inhibit growth through a so-called gain-of-function mutation. Peng *et al.* (1999) found that the *Rht-B1b* and *Rht-D1b* dwarfing alleles each contained a point mutation that introduced a stop codon into a conserved region known as the DELLA domain, which is present near the N-terminus of the protein. Peng *et al.* (1999) proposed that, in *Rht-B1b* and *Rht-D1b*, translation of the protein might restart after the introduced stop codon, resulting shorter proteins in which part of the DELLA domain has been lost. These proteins would then be resistant to GA-induced degradation. As yet, this proposal has not been confirmed experimentally, and there are many uncertainties still to be resolved about the *Rht* genes. For example, two further dwarfing genes, *Rht-B1c* and *Rht-D1c* (formerly known as *Rht3* and *Rht10*, respectively), produce a more severe dwarf phenotype than *Rht-B1b* and *Rht-D1b*. As their name indicates, *Rht-B1c* and *Rht-D1c* are allelic to *Rht-B1b* and *Rht-D1b*, respectively, but it is not known which mutations they contain and how these might result in more severe dwarfing. The rice dwarfing gene arose as a spontaneous mutation in the Taiwanese *indica* strain woo-gen. The resulting strain dee-geo-woo-gen was used in breeding programs in eastern Asia to produce many of the high-yielding semidwarf cultivars grown today. The *sd1* allele in dee-geo-woo-gen contains a 383-base-pair deletion, which introduces a stop codon so that a truncated, inactive enzyme would be produced. Remarkably, semidwarf lines produced independently by mutagenesis, such as the *japonica* varieties Calrose 76 and Reimei, were found also to contain mutations at the *sd-1* locus. Thus, the ideal combination of short stature and high yields provided by *sd-1* mutants has ensured that these alleles have been selected consistently despite the availability of numerous other dwarfing genes in rice, many of which affect GA biosynthesis.

The induced mutations have a great potential to improve any crop species or to create desirable genetic variability, therefore induced mutation is considered as easy, rapid and effective tool of plant breeding. Artificial mutation may be induced following treatment with certain physical or chemical agents called mutagens. In most of the cases, only six mutagens, namely, X- rays, gamma rays, neutron in physical mutagens and EMS, sodium azide and MMS in chemical mutagens are commonly used to create genetic variability in plant improvement. The idea of producing mutation artificially and using it for breeding purposes was clearly started as early as 1901 by De Vries. Induced mutagenesis has been used as a

supplement to conventional breeding procedures ever since evidences on induction of heritable changes in animals (*Drosophila*) and plants (maize and barley) with X-rays were presented by Muller (1927) and Stadler (1928), respectively. They provided the first scientific proof that ionizing radiations like X-rays can enhance the frequency of mutations in animals as well as in plants. Subsequently, studies by Auerbach and Robson (1947) introduced chemicals in the field of mutation breeding. By mutations, plants can be genetically modified and improved in a way similar to traditional breeding methods. The first and the most extensive study involving deliberate selection of mutations in a quantitatively inherited character following X-ray irradiation comes from Gregory's work (1955, 1956(a&b), 1965 and 1968) on peanuts. He presented data to support the hypothesis that as the magnitude of phenotypic effect of the mutation decreases, the frequency of mutant plants increases. Gradually, it has been recognized that micromutation might play an important role in plant breeding, and studies on this aspect for different quantitative characters in mutagen treated populations assumed importance. The parent material for mutation breeding programme can be a recently released cultivar or a promising line. Stubbe (1937) described the small mutations in higher plants for the first time, whereas Knapp and Schreiber (1939) gave clear suggestions for the utilization of micromutations in plant breeding.

The period after 1950 has witnessed many useful mutations for both qualitative and quantitative traits in important crops, especially food crops. During this period, not only the number of released mutant crop varieties increased greatly but the proportion of varieties developed through cross breeding using induced mutants also increased tremendously indicating the recognition of induced mutants used as parents in the breeding programmes by the breeders (Leenakumari et al., 2000). The success achieved with mutation breeding techniques in major crops like rice, wheat and barley during the last half a century has proven that it is no longer a controversial breeding methodology, but should be considered as an important technique to complement conventional breeding technology (Sharma, 1986). Pure and healthy seeds are generally used as the breeding material. Seeds have the advantages over the other plant propagates; because they can be mutagenised in different physical environments, can be desiccated, soaked, heated or frozen or can tolerate high pressure and vacuum conditions. There are more than 2,252 cultivars obtained either as direct mutants or

derived from the crosses have been released worldwide in 50 countries (Maluszynski and Ahloowalia 2000) in a number of crop species. Out of the total varieties released in the world 60% were released from 1985 onwards. Most of the mutant varieties were released particularly in China (26.8%); India (11.5%); USSR and Russia (9.30%) (Ahloowalia et al., 2004). Many induced mutants were released directly as new variety, other were released as parent to drive new variety. The maximum number of varieties has been developed in rice followed by barley and wheat through mutation breeding. Discovery of spontaneous mutants or sports by observant growers has been an important means of cultivar improvement in many fruit crops such as apples and citrus. For example, the apples 'Royal Gala' and 'Imperial Gala' are natural mutations of 'Gala'. In kiwifruit, it is more difficult to trace the source of fruit variants because of the replacement cane pruning system. Unusual fruit found in the packing shed one season may not reappear on the vine the following season if the mutation occurred on a single cane removed during normal winter pruning. Desired improvements/differences would be difficult to detect on single canes. Nevertheless, natural mutations of 'Hayward' kiwifruit have been observed in orchards in New Zealand. Gamma irradiation of dormant budwood has been used in New Zealand in an attempt to induce useful mutations in 'Hayward'.

Mutation in Reverse Genetics

The 21st century is considered as an era of biotechnology and information technology. In the era of biotechnology the genetic engineering has been progressed and strengthened through the discovery of array of molecular techniques viz., PCR, antisense RNA technology, terminator and verminator, genomics and proteomics etc. With the onset of these tools and techniques, a new and innovative technique viz. TILLING (Targeted Induced Local Lesions in the Genome) was developed which will be a potential technology in the reverse genetics. Reverse genetics aims to identify the function of a gene with known sequence by phenotypic analysis of cells or organisms in which the function of this gene is impaired. Commonly used strategies for reverse genetics encompass transposon mutagenesis (Tissier *et al.*, 1999) and RNA-mediated gene silencing or RNA interference (Voinnet, 2002). Most of the genes of an organism are known from sequence, but most of the phenotypes are obscure. Thus, reverse genetics has become an important goal for many biologists. However, reverse-genetic methodologies are

not similarly applicable to all organisms. In the general strategy for reverse genetics that is called TILLING, traditional chemical mutagenesis followed by high-throughput screening for point mutations. TILLING promises to be generally applicable. Furthermore, because TILLING does not involve transgenic modifications, it is attractive not only for functional genomics but also for agricultural applications.

Reverse genetics has become an important goal for many biologists, and new technologies are in great demand (Nagy *et al.*, 2003). Large-scale DNA sequencing projects have changed the way that biology is performed. The traditional pursuit of a gene starting with a phenotype has given way to the opposite situation: most of the genes are known from sequence, but most of the phenotypes are obscure. However, unlike genomic technologies such as DNA sequencing and BLAST searching, reverse-genetic methodologies are not similarly applicable to all organisms. For example, T-DNA insertional mutagenesis has turned the problem of obtaining a gene knockout into an *in silico* procedure for >70% of *Arabidopsis* genes (Alonso *et al.*, 2003), but no comparable resources exist for rice (*Oryza sativa*) or maize (*Zea mays*), despite the increasing availability of high-coverage genomic sequence. RNAi-based silencing is an exciting strategy for reverse genetics (Waterhouse *et al.*, 1998); however, throughput is limited by the difficulty of delivering siRNAs to target loci. Furthermore, the promise of using these reverse-genetic technologies for crop improvement is hampered by genetically modified organism issues.

In TILLING, traditional chemical mutagenesis is followed by high-throughput screening for point mutations. Because of the wide use of chemical mutagenesis for forward-genetic screens in many organisms, TILLING promises to be generally applicable. TILLING is no different from traditional mutation breeding as far as the organism is concerned, so genetically modified organism issues do not arise. This makes TILLING an attractive strategy not only for functional genomics, but also for agricultural applications.

The impetus for TILLING arose from a graduate student's frustration with the limitations of reverse-genetic methods available for *Arabidopsis* in the late 1990s. The original TILLING method used a commercial denaturing HPLC (DHPLC) apparatus for mutation discovery. However, this method would not scale up easily, and so alternative technologies are required. A method for enzymatic mismatch cleavage described by Tony Yeung

seemed particularly attractive (Oleykowski *et al.*, 1998). The LI-COR gel analyzer system (Lincoln, NE) (Middendorf *et al.*, 1992) is ideally suited for this application. By mid-2001, robust protocols and software to begin a TILLING production operation with mutagenized *Arabidopsis* populations have been established (Colbert *et al.*, 2001).

For TILLING *Arabidopsis thaliana* (Fig. 1), seeds were mutagenized by treatment with ethylmethanesulfonate (EMS). The resulting M₁ plants are self-fertilized, and M₂ individuals are used to prepare DNA samples for mutational screening, while their seeds are inventoried and sent to the *Arabidopsis* Biological Resource Center (ABRC) for eventual distribution. The DNA samples are pooled and arrayed in microtiter plates, and the pools are amplified using gene-specific primers. Amplification products are incubated with the CEL I endonuclease, a member of the S1 nuclease family of single strand-specific nucleases (Oleykowski *et al.*, 1998). CEL I cleave to the 3' side of mismatches and loop outs in heteroduplexes between wild-type and mutant DNA while leaving duplexes intact. Cleavage products are electrophoresed using the LI-COR gel analyzer system, and a standard commercial image processing program is used to examine the gel readout. Differential double-end labeling of amplification products allows for rapid visual confirmation because mutations are detected on complementary strands and so can be easily distinguished from amplification artifacts. Upon detection of a mutation in a pool, the individual DNA samples are similarly screened to identify the individual carrying the mutation. This rapid screening procedure determines the location of a mutation to within ± 10 bp for PCR products that are 1-kb in size. For the current mutagenized *Arabidopsis*

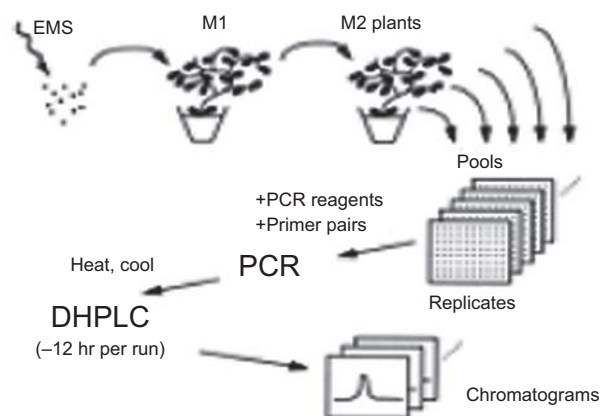


Fig. 1: Schematic depicting the TILLING strategy applied to *Arabidopsis*

populations with a density of 1 mutation per 235 kb, or approximately 4 point mutations per 8-fold pool gel representing 768 plants (Greene *et al.*, 2003).

A key advantage of high-throughput TILLING over competing methods is that the approximate position of each detected mutation is inferred from the size of the fragment, which greatly facilitates subsequent sequencing. Furthermore, the double-end labeling strategy provides confirmation within the pool screen, and further confirmation comes from identifying the same fragments in tracking down individuals. Therefore, sequencing is done with near certainty that a mutation exists within a small interval. Examination of a sequencing gel trace in the predicted location suffices to identify the mutated base and the substitution, and we use Sequencher trace analysis software (Gene Codes, Ann Arbor, MI) to facilitate this step. Finally >3,000 *Arabidopsis* mutations were identified by this procedure, typically using the readout from only the strand in which the primer is closer to the detected mutation. By contrast, methods that do not provide an approximate location for a detected mutation, such as DHPLC, require that the full amplified segment be interrogated by sequencing, and for a 1-kb segment this would require multiple runs to be carefully scrutinized. Detection of heterozygotes under such circumstances can be challenging, especially when peak heights vary, and false positives will greatly exacerbate this problem. The increasing use of such mutations in evolution, crop improvement using conventional induced mutagenesis and high throughput screening (TILLING) will highly be appreciated among the breeders and biotechnologists in 21st century.

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Seed Oil Quality of *Perilla frutescens* (L.) Britt. Genotypes

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Perilla frutescens accessions collected from North East Hill Region of India were analyzed for their seed oil, fatty acids and seed protein contents. The seed size of the accessions was diverse, and hundred seed weight averaged 0.130 g with a range of 0.230g to 0.052g. The accessions IC419706 and IC419477 were identified for possessing more than 50% oil in the seed. Ratio of omega-6 to omega-3 fatty acid ranged from 0.52 to 0.21. Accessions IC374494, IC369352, IC369349, IC006440, IC521292 and IC374609 were having high linolenic acid content (>63%) in the oil. Four other collections, IC334313, IC521287, IC374543 and IC335402 showed more than 23% linoleic acid against average value of 19.70%. The saturated fatty acid concentration of oils varied within a narrow range of 8-11% of the oil with palmitic acid contribution of about 75%. IC374543 and IC419475 had more than 9% palmitic acid. Significant positive relationship was observed between seed size and oil content of the seed.

Key Words: *Perilla*, Germplasm, Oil, Fatty acid, Protein

Introduction

Perilla frutescens (L.) Britton, commonly known as beefsteak plant is an annual herb of the mint family (Lamiaceae) and a native of East Asia (Fig. 1). It is a self pollinated species and is grown as a traditional crop in China, India, Japan, Korea, Thailand and other Asian countries. It has been used for centuries in oriental medicine as an antiasthmatic, antibacterial, antidote, antimicrobial, antipyretic, antiseptic and antispasmodic. In modern days, this plant finds its importance for its nutritious cooking seed oil as it contains about 64% linolenic acid, an omega-3 fatty acid (Shin and Kim, 1994) which is generally absent or present in very low proportion in polyunsaturated fatty acids (PUFA) component of different oilseed crops which is used for edible purposes. Omega-6 and omega-3 fatty acid, the two PUFA components, are transformed into variety of complex unsaturated fatty acids known as eicosanoids. These eicosanoids plays an important role in the moment-by-moment regulation of metabolism at the microcellular and tissue levels (Wysong, 1990). The Alpha linolenic acid (ALA) acts as precursor for conversion into long chain omega-3 PUFAs (LC omega-3 PUFA), namely eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA). The beneficial effect of PUFA is mainly due to EPA and DHA. By increasing the dietary ALA, EPA and DHA concentration increases the plasma phospholipids which impart beneficial health effects. (Indu and Ghafoorunissa, 1992).

The PUFA content has been reported to be beneficial when, omega-6 and omega-3 fatty acids are present in

right proportions, i.e. 3:1 to 5:1. (Simopoulos and Cleland, 2003). Eicosanoids which is produced from the excess omega-6 fatty acids cause platelet aggregation and clot formation, whereas, the eicosanoids produced from the omega-3 fatty acids tend to reverse these effects by increasing clotting time and decreasing coagulation factors including viscosity of blood. Therefore fish oils and other oil sources viz., flax seed oil, walnuts, soybean etc. having high proportions of omega-3 fatty acids may included in the diet. Omega-3 fatty acids especially those from *Perilla frutescens* and fish oil have been shown to enhance brain functions and maintain the blood brain barrier which increases longevity (Crawford, 1987).

Due to various beneficial oil properties, different research programs have been initiated to build up genetic stocks of *Perilla frutescens* crop through introduction and collection activities at the National Bureau of Plant Genetic



Fig. 1: *Perilla frutescens* var. *frutescens*

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Resources (NBPGR), New Delhi. Systematic evaluation programs are also undertaken for the selection of promising and unique genotype for use in crop improvement programmes. In the present study, fifty *Perilla frutescens* germplasm accessions collected from North-Eastern Hill Region of India were grown at NBPGR Regional Station, Shillong and analyzed for its seed oil quantity/quality and seed protein content.

Materials and Methods

Plant Material

Fifty collections of *Perilla frutescens* germplasm were made during 2000-2005 from different parts of North Eastern Hill Region of India (Table 1). These collections were grown in single observational rows at Barapani farm (Altitude 1000 m) Shillong (India) during *Kharif* season in 2005. The plants were spaced 50 cm from row to row and 20 cm within the row. At planting FYM was applied in rows and basal dose of 40N, 50P, and 40K kg/ha was applied. No top dressing of nitrogenous fertilizer was given. Observations were recorded on randomly chosen five plants in each accession. Mature seeds were taken for the count of hundred seed weights and also for chemical analysis.

Total Oil Content of Seed

The *Perilla frutescens* seeds were dried to 4-5% moisture level in oven at 108°C for 16 to 18 hr. The oil content of the seed samples were determined by non-destructive method using a Newport NMR analyzer (Model-4000) from Oxford Analytical Instruments Ltd., UK, equipped with 40ml coil assembly. The instrument was kept in a room of constant temperature (23-25°C). Pure *Perilla* seed oil required to calibrate the instrument, was extracted by solvent extraction method followed by Madsen (1976). The instrument was run by adjusting audio frequency (AF) gain of 400 and radio frequency (RF) current of 225 μ A with gate width of 1.0 gauss. The NMR responses (signal/mass) of the seed samples were compared to NMR response of pure oil (100%) for obtaining oil percentage of the *Perilla* accessions.

Fatty Acid Analysis by Gas Liquid Chromatography (GLC)

Samples of *Perilla frutescens* seeds were freshly ground (Remi homogenizer) to get 40 mg oil extracted with 10ml solvent mixture consisting of chloroform: hexane: methanol (8 : 5 : 2 v/v/v). The extracts obtained were dried at 60°C in nitrogen gas for 30 min. Methyl esters

Table 1. Habitat of *Perilla* accessions

S.No.	Collection No.	IC No.	Habitat	S.No.	Collection No.	IC No.	Habitats
1	H-1099	IC521285	Garro Hills	26	DRLT-1524	IC416861	Arunachal Pradesh
2	H-1748	IC003908	Arunachal Pradesh	27	RD-74	IC374593	Mizoram
3	FDI/RS-134	IC419477	Arunachal Pradesh	28	H-1796	IC003942	Garro Hills
4	RD-134	IC374513	Mizoram	29	FDI/RS-05	IC419710	Arunachal Pradesh
5	BDS-1645	IC006442	Arunachal Pradesh	30	GP-178	IC521287	Meghalaya
6	H-3639	IC204185	Arunachal Pradesh	31	FDI/RS-243	IC419598	Arunachal Pradesh
7	BDS-1647	IC006444	Arunachal Pradesh	32	USK-419	IC330445	Manipur
8	NH-6/10	IC521288	Nagaland	33	BDS-1643	IC006440	Arunachal Pradesh
9	FDI/RS-250	IC419606	Arunachal Pradesh	34	H-1143	IC521283	Meghalaya
10	RS/SB/BP-59	IC369349	Mizoram	35	RS/SB/BP-61	IC369352	Manipur
11	H-556	IC521286	Mizoram	36	—	IC 526643	Manipur
12	BDS-1646	IC006447	Arunachal Pradesh	37	BDS-837	IC521290	Tripura
13	RD-117	IC374494	Mizoram	38	H-529	IC521292	NC Hills
14	RS-012/96	IC204210	Mizoram	39	RS-72/99	IC335408	Mizoram
15	BDS-1646	IC006443	Arunachal Pradesh	40	RS-66/99	IC335402	Mizoram
16	BDS-1649	IC006446	Arunachal Pradesh	41	H-621	IC521284	Garro Hills
17	H-1812	IC003955	Garro Hills	42	RD-89	IC374609	Mizoram
18	RD-71	IC374590	Mizoram	43	FDI/RS-046	IC419706	Arunachal Pradesh
19	BDS-1644	IC006441	Arunachal Pradesh	44	H-2216	IC012640	NC Hills
20	H-664	IC521282	Garro Hills	45	FDI/RS-132	IC419475	Arunachal Pradesh
21	FDI/RS-212	IC419564	Arunachal Pradesh	46	VRB-MA-2029	IC521293	Uttarakhand
22	H-1756	IC003942	Garro Hills	47	H-746	IC521291	Garro Hills
23	RD-29	IC374543	Mizoram	48	USK-415	IC330441	Manipur
24	H-3944	IC211608	Arunachal Pradesh	49	H-794	IC521289	Garro Hills
25	FDI/AP-02	IC334313	Arunachal Pradesh	50	H-1696	IC003865	Mizoram

of oil samples were prepared according to the method of Neff *et al.* (1994) with slight modifications as applied for cruciferous species by Mandal *et al.* (2002). 1 µl of the hexane extract was injected into a highly polar HP Innowax capillary column of 30m length (inner diameter: 0.32 mm, film thickness: 0.5 µm, split: 1:80). A Hewlett Packard gas chromatograph, model 6890 equipped with flame ionization detector (FID) was used. The injector and detector temperatures were 260°C and 275°C respectively. Oven temperature was programmed from 150°C holding at 1 min. to 210°C at the rate of 15°C/min., followed by 210°C to 250°C at the rate of 5°C/min. for 12 min. Peaks of fatty acid methyl esters were identified by comparing their retention time with that of the known standards run under similar separation conditions. Peak integration was performed applying HP3398A software.

Protein Content

Nitrogen content of the *Perilla* seeds was determined by conventional Kjeldahl method with Kjeltac analyzer (Model-2300) from Foss Tecator, Sweden. Factor 6.25 was used to convert nitrogen to protein.

Scanning of Samples by Near Infrared Spectroscopy

About 5g intact seed were uniformly placed in a small ring cup (φ 3.8 cm) for scanning on a Monochromator NIR Systems, Model TR-3712-6 (Foss Tecator Near Infrared Analyzer System: Silver springs MD 20904, U.S.A.) and reflectance spectra (log 1/R) from 400 – 2500nm were recorded at 8 nm intervals. The log (1/R) spectra were transformed into their second derivative and DE-trend scatter correction (Barnes *et al.*, 1989) was

computed at gaps of 4 nm and smoothing over segments or length 4nm. Calibration was performed by using modified partial least square (MPLS) regression on the range from 1100 to 2498 nm. Cross validation was used to prevent over fitting (Shenk and Westerhaus, 1993). All procedures were developed by using WIN-ISI software version 3.10 (Infrasoft International, Port matida PA, USA).

Results and Discussion

Seeds of *Perilla frutescens* accessions were comparatively small in size. Hundred seed weight were found varied within the range of 52mg to 230mg. Seed oil contents determined at 4% moisture level were between 51.66 and 30.10% with a mean of 45.44%. Significant positive association was found between seed weight and seed oil concentration. Seven high oil-containing (>48%) accessions with their fatty acid profile and other seed quality are shown in Table 2. Present accessions contain about 18.6% protein (%N X 6.25) in the seeds. Variation of seed protein was in the range of 22.8 to 15.3%. Protein trait of the seed bears no relationship either with oil or size. The combine oil-protein content of the seed was found varied within the range of 70.4 to 48.5%.

Palmitic, stearic, oleic, linoleic and linolenic are the five fatty acids found present in *Perilla frutescens* seed oil. The average individual fatty acid values as established for the present accessions were 7.32%, 2.50%, 10.54%, 19.70% and 59.78% respectively. Linolenic acid (omega-3 fatty acid) predominated in the seed oil and contributed from 66.06 to 53.78%. Linoleic (omega-6) acid, the other component of polyunsaturated fatty acid (PUFA) contributed to the extent of 28.02 to 13.38%. Contribution

Table 2. High oil containing *Perilla frutescens* accessions

Accession No.	Protein (%)	Palmitic (%)	Stearic (%)	Oleic (%)	Linoleic (%)	Linolenic (%)	Omega-6/omega-3 ratio
IC003908	22.01	6.78	2.68	12.94	22.74	54.87	0.41
IC330441	20.94	7.49	2.98	9.97	19.10	60.46	0.32
IC374494	17.07	7.35	3.45	8.53	14.62	66.06	0.22
IC369352	20.04	6.89	2.50	9.50	18.96	63.80	0.31
IC374609	18.29	7.52	2.10	10.39	16.30	63.69	0.26
IC374543	18.37	9.00	1.71	10.11	23.18	55.67	0.42
IC419475	18.62	9.01	2.11	11.42	22.35	54.91	0.41
IC335408	15.27	9.01	1.76	7.94	21.53	59.81	0.35
IC334313	15.30	7.37	2.17	8.66	27.00	53.78	0.52
IC521287	18.23	8.20	2.30	10/32	24.27	54.91	0.44
IC374543	18.37	9.33	1.71	10.11	23.18	55.67	0.41
IC335402	16.43	8.78	2.17	8.73	23.02	57.30	0.40
IC369349	20.23	7.19	4.58	8.19	15.43	63.39	0.24
IC006444	18.42	7.54	1.48	13.89	16.41	60.68	0.27

of oleic acid, the only monounsaturated fatty acid (MUFA) component, varied from 13.89 to 7.57%. Palmitic and stearic acid, the two saturated fatty acid components accounted for 11.7 to 8.0 % of the total oil content. Among this palmitic acid contributed to the range of 9.01 to 6.42% and stearic acid to the range of 4.58 to 1.35%. Significant negative correlation was observed between oil content of the seed and its palmitic acid concentration. Relationships between different fatty acids are shown in Table 3. Highly significant and negative relationship was observed between two components of PUFA.

Present accessions produced seed, which were small in size. Some Exotic collections (EC216365, EC216371) from Republic of Korea, studied earlier (Sharma *et al.*, 1989) at our laboratory gave larger sized seeds with hundred seed weight of 400 mg. IC006440, IC521291, IC419706, IC521284, IC006446, IC521293, IC419475, IC521285 and IC521292 were the nine accessions identified from the present population for producing larger sized seeds, weighing more than 180 mg to 230mg. IC003908, IC369349, IC374590, IC419564, IC374543, IC416861 and IC374593 were the other seven accessions that produced very small sized seeds with hundred seed weight of less than 100 mg.

Oil value for *Perilla* seeds have been reported from 38.6 to 47.8% by Shin and Kim (1994). More than ninety percent population falls within this range. IC419706 and IC419477 with seed oil content of 51.6% and 50.4% respectively were considered as high oil yielding genotypes. For confirmation of their rich quality value, NIRS (Near infrared reflections spectroscopy) based analytical techniques were applied.

NIRS is a multi trait technique being used for rapid, comparative and accurate estimation of quality trait of intact seed (Tkachuk, 1981; Van Deynze and Pauls, 1994; Velasco and Becker 1998a, 1998b). The technique has been considered an empirical method because calibration equations (Predicting software) have to be obtained prior to analysis. Calibration equation generally developed with different lot of samples with their respective constituent values determined by primary and conventional method of analysis. In present case, because of non-availability of sufficient accessions, the respective calibration equations were developed with same samples but with omission of corresponding value rich lines. Prediction statistics of different NIR equations developed with full range of samples are given in Table 4. Equations are in use for ascertaining the quality values of new *Perilla*

Table 3. Relationship between quality traits in *Perilla frutescens* accession

	Weight	Oil	Protein	Palmitic acid	Stearic acid	Oleic acid	Linolenic acid
Oil	0.697**						
Protein	0.102	0.087					
Palmitic acid	-0.365	-0.492**	-0.256				
Stearic acid	-0.069	-0.062	0.119	-0.225			
Oleic acid	0.170	0.164	0.299	-0.295	-0.190		
Linoleic acid	-0.258	-0.326	-0.150	0.399**	-0.428**	0.007	
Linolenic acid	0.279	-0.062	0.005	-0.382	0.167	-0.374**	-0.866**

** Significant at $p = 0.01$

Table 4. Calibration statistical data for NIRS analysis of intact *Perilla* seeds for total oil (% oil in seed), protein (% protein in seed), individual fatty acids (% of total oil)

Trait	Range	Mean $\pm$ SD	SEC	R ²	SECV	1-VR
Oil	51.66-30.10	45.33 $\pm$ 3.84	0.379	0.99	0.912	0.944
Protein	22.83-15.27	18.59 $\pm$ 1.49	0.449	0.910	1.138	0.446
Palmitic acid	9.00-6.42	7.32 $\pm$ 0.72	0.219	0.894	0.831	0.200
Stearic acid	4.58-1.35	2.41 $\pm$ 0.605	0.260	0.815	0.662	-0.134
Oleic acid	13.89-7.57	10.53 $\pm$ 1.51	0.606	0.841	1.714	-0.272
Linoleic acid	27.00-13.38	19.69 $\pm$ 2.62	0.850	0.890	3.023	-0.320
Linolenic acid	66.06-53.78	59.89 $\pm$ 2.840	1.029	0.869	3.226	-0.285

Where, SD= Standard deviation,

R² = Squared co-efficient of multiple determination in calibration. In cross validation it is estimated as 1 minus variance ratio (1-VR),

SEC= Standard error of calibration.

SECV = Standard error of cross validation in modified partial least squares regression

samples, generated under present programme. Equations will get enriched with inclusions of more and more quality rich *Perilla* genotypes in calibration set. Three trait specific histogram showing distribution of number of accessions with low to high value within total variation of trait with respect to oil, protein, and linolenic acid are shown in Figure 2 to Figure 4 respectively.

Perilla frutescens oil is very similar to flax oil with respect to fatty acid composition. Like flax oil, *Perilla frutescens* also showed omega-6 fatty to omega-3 fatty acid ratio of 0.33:1 and omega-3 rich PUFA composition of about 79.6%. Coefficients of variation for omega-6 and omega-3 acids were 13.34% and 4.78% respectively. Omega-6 to omega-3 ratio values found varied in the range of 0.55 to 0.22 and mean value was 0.32. *Perilla* oil has been reported to impart number of beneficial effects because of the presence of higher proportion of omega-3 acid in oil. It is still not known exactly what particular ratio among the two components of PUFA can

serve more beneficial way. Due to the presence of low concentration of oleic acid the shelf life is very short and it should be refrigerated in order to obtain the benefits of omega-3 fatty acid of the oil. Fatty acid profile of *Perilla frutescens* oil along with other quality traits that were established in the present study can serve very important information for the future application of *Perilla frutescens* oil.

Rich diversity of *Perilla frutescens* exists in the Indian Himalayas, where it is grown as a backyard crop. It has a great potential as an oilseed crop as evident by the present study, though it has not been grown commercially. Therefore, there is a need to fully investigate the agronomical potential of this species for desirable traits. More germplasm needs to be collected and evaluated for inclusion of this crop in the low input farming system coupled with value addition in the Indian Himalayas.

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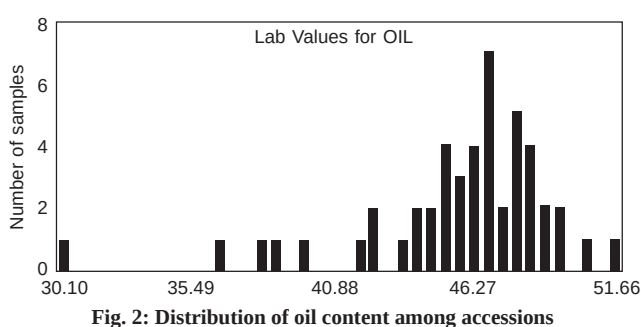


Fig. 2: Distribution of oil content among accessions

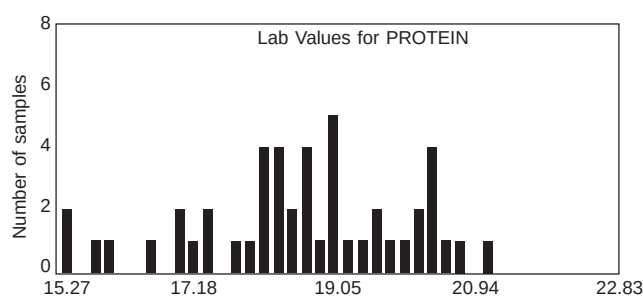


Fig. 3: Distribution of protein content among accessions

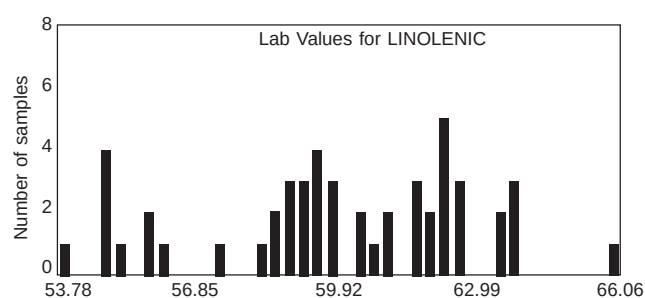


Fig. 4: Distribution of linolenic acid content among accessions

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Evaluation Studies on Some Walnut Genetic Resources in Himachal Pradesh

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Thirty two genetically diverse walnut accessions were characterized and evaluated for the period of three years (2006-2008). All the accessions bore nuts in the terminal position while all but one possessed satisfactory kernel flavour. More than half the accessions studied exhibited 'spreading' tree shape, medium-sized leaves, elliptic leaflets, large catkins, protandry, 'round' nuts, 'medium' intensity in shell colour, amber-coloured kernels and 'medium' productivity. Highest Coefficient of Variation was observed for kernel weight (38.50%), number of staminate flowers/inflorescence (35.42%), shell weight (33.95%), and nut weight (32.88%) indicating that there is enough potential for exploiting the variability present in these characters. The huge variability displayed might be due to the presence of two related species, material from diverse geographical origin and seedling origin of indigenous materials. Highly significant and positive phenotypic correlation between nut weight and its associated traits like nut width, nut length, shell weight, shell thickness and kernel weight was observed. High productivity was recorded in nine accessions, but analysis of nut traits, and other important traits revealed that two accessions namely IC019378 and IC020108 may merit for popularization. Promising acc. were also identified for individual traits affecting nut quality which can serve as donors in breeding programme.

Key Words: Walnut, Characterization, Evaluation, Genetic resources, *Juglans regia*

Introduction

Walnut grows as a wild species in mixed broadleaves forest from Southern Europe to India. It is found in the Balkan area (Hungary, former Yugoslavia, Bulgaria, Albany), Caucasus, Asia Minor (Turkey, North Iran and Iraq), central Asia (the North limit in Kyrgyzstan), having its eastern limit in the North of India (Hemery, 1998). Central Asia and more specifically the mountains placed at West of Himalayan chain in the Kashmir, Tajikistan and Kyrgyzstan are considered to be the centre of origin of *J. regia* L. The genus *Juglans* includes 21 species among which *Juglans regia* is the important one and being cultivated commercially in many countries. Walnut is one of the most important nut crops in India, and its nearest relative *J. sigillata* Dode is concentrated in Eastern Himalayan region of India and China. In India, it is mostly grown in homestead level, although few orchards are coming up recently. Jammu & Kashmir is principal walnut growing state, however, Chamba in Himachal Pradesh, and Chakrata hills of Uttarakhand are also famous for walnut production. Walnuts are heterozygous, monoecious, self fruitful as well as cross compatible (also with other species) due to the existence of varying levels of dichogamy and pollination through wind, thus great variability exist in natural population. NBPGR Regional Station Shimla has augmented walnut germplasm through introduction of cultivars from abroad and indigenous material from various parts of Himalaya. Present study deal with work

on characterization and evaluation of 32 accessions of walnut and also identifies some accessions and correlated traits useful in breeding.

Materials and Methods

The study was carried out at field gene bank of NBPGR Regional Station, Phagli, Shimla, Himachal Pradesh (31° 05' 924" N latitude, 77° 09' 580" E longitude; 1924 m MSL). Thirty two genetically diverse walnut accessions comprising of 16 exotic (from four countries) and 16 indigenous were taken for study. All the plants were grafted over seedling *J. regia* rootstock and were of 15-20 years old. Observations were recorded on one or two trees per accession for qualitative characters like tree shape, leaf size, leaflet shape, staminate inflorescence (catkin) size, pistillate inflorescence position, dichogamy, nut shape, shell softness, shell surface, shell colour intensity, kernel colour and kernel flavour and quantitative traits like leaf length, number of staminate flowers/inflorescence, date of bud break, start and end of flowering, fruit maturity and leaf fall, nut length, nut width (cheek diameter), nut weight, shell thickness, kernel weight and kernel per cent as per descriptors developed by National Bureau of Plant Genetic Resources (Mahajan *et al.*, 2002). Fruit data were recorded from randomly selected ten nuts from each accession. Pooled mean values of three years (2006-2008) data were taken to draw the conclusion. Variability parameters, frequency distribution and correlation coefficients were worked out and potential

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accessions were identified.

Results and Discussion

Many of the descriptor states mentioned by Mahajan *et al.* (2002) (Table 1) were represented in the accessions studied indicating the presence of large variability for qualitative characters studied. All the accessions bore

Table 1. Frequency distribution of various qualitative characters in 32 walnut accessions studied

Descriptor/descriptor state	Absolute frequency	Relative per cent
Tree shape		
Spreading	27	84.37
Upright	4	12.50
Drooping	1	3.12
Leaf size		
Small	3	9.38
Medium	22	68.75
Large	7	21.87
Leaflet shape		
Elliptic	18	56.25
Broad elliptic	14	43.75
Male inflorescence size		
Small	3	9.38
Intermediate	12	37.50
Large	17	53.12
Female Inflorescence position		
Terminal	32	100
Dichogamy		
Protandrous	16	50.00
Protogynous	3	9.38
Homogamous	13	40.62
Nut Shape		
Round	16	50.00
Oblong	6	18.75
Ovate	4	12.50
Elliptic	5	15.63
Cordate	1	3.12
Shell thickness		
Soft	8	25.00
Intermediate	12	37.50
Hard	9	28.13
Very hard	3	9.37
Shell surface		
Smooth	14	43.75
Light corrugated	12	37.50
Highly corrugated	6	18.75
Shell colour intensity		
Medium	22	68.75
Light	4	12.50
Dark	3	9.38
Very dark	3	9.38
Kernel colour		
Extremely light	1	3.12
Light	5	15.63
Amber	20	62.50
Dark amber	6	18.75
Kernel flavour		
Satisfactory	31	96.87
Unsatisfactory	1	3.12
Productivity status		
Low (<30 kg/plant)	5	15.63
Medium (30-50 kg/plant)	18	56.25
High(>50 kg/plant)	9	28.13

pistillate flowers in the terminal position while all but one possessed satisfactory kernel flavour. Apart from this, more than half the accessions studied exhibited 'spreading' tree shape, medium-sized leaves, elliptic leaflets, large catkins, protandry, round nuts, 'medium' intensity in shell colour, amber-coloured kernels and 'medium' productivity. Nevertheless, more than ten accessions had broad elliptic-shaped leaflets, medium-sized catkins, concurrent pollen dehiscence and stigma receptivity and 'intermediate' shell thickness. A note can be made of shell colour, which is of market concern in the regional level, although for international export this may not be a concern, due to exporting kernel only. Light coloured shells are highly preferred although 'medium' intensity in shell colour is also acceptable. In the present study, accessions namely IC019371, IC019378, IC020108 and EC038653 produced light coloured nuts. Capacity to produce light coloured kernel is strongly cultivar dependant but its expression includes environment component also, for instance, many cultivars which produce acceptable light kernels in cooler climates darken excessively when grown in the warmer areas (McGranahan and Leslie, 1990). Only six accessions exhibited light kernel colour while 20 exhibited amber coloured kernels. According to Bhat *et al.* (1992), light amber colour is a not serious handicap in the market, but dark and black kernels are least preferred. Smooth surfaced nuts are common in exotic accessions but rare in indigenous accessions. High productivity was recorded in nine accessions (EC024507, EC024582, EC026860, EC026891, EC027484, EC035719, EC038837, IC019378 and IC020108) and medium productivity in 20 accessions.

Perusal of Table 2 revealed large variation in quantitative traits studied in terms of range and coefficient of variation (CV). Date of start of bud break varied from 16th February (EC043408) to 10th April (EC036744) with two third of the accessions break their dormancy during second fortnight of March. Date of bud break has importance since it is directly linked with leafing date and flowering date. If bud break is late (i.e. after 15th March), accessions most probably escape from frost injury. Date of start of flowering (male/female) ranged from 18th March (IC019376) to 17th April (EC036744) while date of end of flowering varied from 24th March (IC019376) to 25th April (EC036744). Although, in most of the accessions, flowering lasts for about ten days, the studied genetic resources had the proficiency of offering pollination for about seven incessant weeks, hence revealing the enormous scope for pollinating those

Table 2. Variability parameters for various quantitative characters in 32 walnut accessions

Sl.No	Accession	Alternate identity	Date of bud break	Start of flowering	End of flowering	Date of fruit maturity	Date of leaf fall	Leaf length (cm)	Staminate flowers /infl.	Nut length (cm)	Nut width (cm)	Nut weight (g)	Shell weight (g)	Shell thickness (mm)	Kernel weight (g)	Kernel percentage
1	EC024507	<i>J. regia</i> - 1	2nd Apr.	9th Apr.	14th Apr.	23rd Aug.	15th Oct.	27.00	76.00	3.34	3.03	10.38	6.12	1.79	3.99	38.14
2	EC024562	Lake English	31st Mar.	4th Apr.	14th Apr.	8th Aug.	5th Oct.	27.60	73.33	2.71	2.93	6.16	3.26	1.28	2.77	45.06
3	EC026860	<i>J. nigra</i> - 1	31st Mar.	14th Apr.	23rd Apr.	7th Aug.	20th Sep.	27.15	56.00	2.07	2.23	4.27	2.88	1.79	1.37	32.49
4	EC026891	Black More	2nd Apr.	6th Apr.	15th Apr.	12th Aug.	16th Oct.	28.37	81.50	3.56	3.19	8.46	4.89	1.49	3.55	42.03
5	EC027484	Turtle-31	29th Mar.	5th Apr.	17th Apr.	14th Aug.	30th Sep.	22.00	84.50	2.86	2.77	6.39	4.15	1.60	2.77	43.73
6	EC031071	<i>J. regia</i> - 2	19th Mar.	25th Mar.	4th Apr.	17th Aug.	20th Oct.	18.13	67.20	3.09	2.69	9.19	5.90	1.78	3.21	34.71
7	EC035719	<i>J. mandshurica</i>	20th Mar.	6th Apr.	21st Apr.	15th Sep.	3rd Nov.	76.67	222.67	2.96	2.25	4.60	3.46	1.63	1.12	24.41
8	EC036744	Sparrow (<i>J. nigra</i>)	10th Apr.	17th Apr.	25th Apr.	19th Sep.	12th Oct.	28.37	45.00	3.29	3.51	12.79	8.96	2.53	3.42	27.29
9	EC036746	Ogden	20th Mar.	28th Mar.	8th Apr.	6th Aug.	26th Oct.	34.82	91.75	3.60	3.08	10.44	5.91	2.10	4.52	43.82
10	EC038748	Orth	29th Mar.	7th Apr.	13th Apr.	14th Aug.	30th Sept.	29.25	80.00	3.82	3.08	9.17	5.62	1.83	3.56	38.31
11	EC036752	<i>J. regia</i> - 3	20th Mar.	30th Mar.	8th Apr.	9th Aug.	11th Oct.	31.50	87.45	3.81	3.13	8.79	5.27	1.56	3.19	36.18
12	EC038653	Bandoni	2nd Apr.	9th Apr.	17th Apr.	15th Aug.	24th Oct.	26.75	74.25	3.80	2.69	7.51	4.78	1.42	2.71	36.04
13	EC038828	Xenia-1	20th Mar.	29th Mar.	10th Apr.	14th Aug.	22nd Sep.	22.83	77.57	3.64	2.97	9.13	5.67	1.63	3.37	37.00
14	EC038836	Hartley	20th Mar.	31st Mar.	8th Apr.	11th Aug.	28th Sep.	20.25	81.00	4.11	3.60	14.11	8.66	1.72	5.43	38.48
15	EC038837	Kx Giant	11th Mar.	25th Mar.	9th Apr.	6th Aug.	15th Oct.	31.10	87.50	4.39	3.87	13.40	9.52	2.30	4.06	30.30
16	EC043408	<i>J. nigra</i> - 2	16th Feb.	21st Mar.	1st Apr.	26th Jun.	4th Oct.	24.50	26.50	2.14	2.31	4.47	3.40	2.12	1.14	25.59
17	IC019369	BDJ-93-3	21st Mar.	31st Mar.	11th Apr.	17th Sep.	27th Oct.	31.10	87.00	3.99	3.80	18.48	11.11	2.38	7.01	38.11
18	IC019371	BDJ-93-7	1st Apr.	6th Apr.	14th Apr.	29th Aug.	30th Sep.	32.00	68.75	2.79	2.84	9.42	6.73	2.00	2.03	21.85
19	IC019376	BDJ-93-15	1st Mar.	18th Mar.	24th Mar.	28th Sep.	2nd Nov.	29.33	96.50	3.71	3.57	14.91	9.18	2.42	5.54	37.17
20	IC019378	BDJ-93-30	24th Mar.	10th Apr.	16th Apr.	22nd Aug.	15th Oct.	25.00	85.50	3.32	2.94	9.06	4.61	1.70	4.25	46.79
21	IC020067	BDJ-108	10th Mar.	31st Mar.	9th Apr.	8th Sep.	24th Oct.	36.80	83.67	3.58	3.16	11.63	6.98	1.93	4.59	40.77
22	IC020068	BDJ-109	1st Apr.	6th Apr.	13th Apr.	1st Sep.	17th Oct.	36.35	83.00	3.22	3.26	11.46	8.45	2.58	2.61	23.04
23	IC020070	BDJ-111	5th Apr.	16th Apr.	21st Apr.	29th Aug.	13th Oct.	30.93	77.00	3.60	3.10	14.84	10.68	2.40	3.65	24.52
24	IC020107	BDJ-148	27th Mar.	16th Apr.	10th Apr.	26th Aug.	24th Oct.	37.70	78.30	4.07	3.41	12.15	7.75	1.76	4.26	34.39
25	IC020108	BDJ-149	24th Mar.	30th Mar.	6th Apr.	10th Sep.	25th Oct.	31.50	81.25	3.97	3.39	13.33	7.29	1.04	5.98	44.87
26	IC020109	BDJ-150	20th Mar.	31st Mar.	9th Apr.	9th Sep.	1st Nov.	29.50	76.00	3.39	2.90	8.77	5.45	2.06	3.20	36.24
27	IC020112	BDJ-153	16th Mar.	31st Mar.	9th Apr.	12th Sep.	28th Oct.	22.67	89.00	2.69	2.73	7.54	5.57	2.03	1.76	24.98
28	IC020114	BDJ-155	22nd Mar.	30th Mar.	7th Apr.	9th Sep.	10th Oct.	26.50	71.00	3.55	3.12	10.93	6.45	1.74	4.14	37.87
29	IC020119	BDJ-160	23rd Mar.	2nd Apr.	5th Apr.	5th Oct.	21st Oct.	31.73	80.00	3.23	3.26	12.25	8.04	2.08	4.08	33.69
30	IC020075	BDJ-116	30th Mar.	9th Apr.	16th Apr.	30th Sep.	21st Oct.	37.33	89.00	3.46	3.51	12.04	7.95	2.14	3.44	29.71
31	IC538531	Sml-1	15th Mar.	31st Mar.	11th Apr.	22nd Aug.	3rd Nov.	33.17	82.83	3.49	3.21	11.96	6.74	1.71	5.09	42.23
32	IC558115	Sml-2	13th Mar.	25th Mar.	4th Apr.	30th Aug.	14th Nov.	33.00	88.67	2.64	2.49	6.35	3.73	1.43	2.35	37.19
							Min.	18.13	26.50	2.07	2.23	4.27	2.88	1.04	1.12	21.85
							Max.	76.67	222.67	4.39	3.87	18.48	11.11	2.58	7.01	46.79
							Mean	30.65	82.18	3.37	3.06	10.14	6.43	1.87	3.57	35.22
							SE	1.72	5.14	0.10	0.07	0.59	0.38	0.07	0.24	1.25
							CV(%)	31.74	35.42	16.30	13.56	32.88	33.95	19.81	38.50	20.00

accessions facing dichogamy problem. Walnut is an anemophilous species, produces heavy pollen that moves over 100 metres and contains dichogamy which lead to autocompatibility (Germain *et al.*, 1973). Forde and McGranahan (1996) stated that predominance of protandry is also appeared in the germplasm collection. Study on dichogamy is important as it will help us to identify the cultivars which need artificial pollination or to be planted with other cultivars whose pollen shedding coincides with formers' stigma receptivity. In western countries, one of the breeding objectives is to get a cultivar with a better overlap of flowering of both staminate and pistillate flowers. About 40% accessions of this study had such concurrent flowering nature.

Date of fruit maturity ranged from 26th June (EC043408) to 5th October (IC020119) with a huge time interval of 102 days. Those accessions which matures early (before the end of first week of August) in the harvest season were EC043408, EC036746 and EC038837 while late maturing (after 27th of August) accessions were IC020119, IC020075 and IC019376. In general, indigenous walnut accessions were late in maturity as compared to exotic accessions. The accession EC043408 took only 91 days from flowering to fruit maturity and matured in late June itself while IC020119 took 183 days for the same. Former accession may form a source for breeding early walnut cultivars.

In the present study, range of phenotypic variability as revealed by highest CV(%) was recorded for kernel weight (38.50) followed by number of staminate flowers/ inflorescence (35.42), shell weight (33.95), and nut weight (32.88) indicating that there is enough potential in utilizing the variability for these characters (Table 2). The nut length varied from 2.07–4.39cm, nut width 2.23-3.87cm and nut weight 4.27-18.48g. Shell weight ranged from 2.88-11.11g, shell thickness 1.04-2.58mm, kernel weight 1.12-7.01g and kernel recovery 21.85-46.79%. Pandey *et al.* (2004) reported range of nut weight varied from 7.68-18.45g in Uttarakhand collections; 6.29-27.16g in J&K collections and 9.18-19.98g in Himachal Pradesh collections. Aslam (1993) reported nut weight variation from 9.36 to 12.96g in case of indigenous selections and 8.45 to 10.46g in exotic cultivars. Pandey *et al.*, (2004) reported highest kernel recovery 66.26 % in 150 walnut accessions collected from J&K. Similar results were also reported by Bhat *et al.* (1992) and Rana *et al.* (2007). Queshi and Dalal (1985) reported 30-35% kernel recovery in seedling walnut population collected from Kinnaur district of Himachal Pradesh. In the present study huge

variability displayed for qualitative and quantitative characters might be due to the presence of two other species which hybridize easily with *J. regia* namely *J. nigra* L. (two accessions.) and *J. mandshurica* Maxim. (one accession.), material from diverse geographical origin and seedling origin of indigenous materials. Such high level of variability for qualitative and quantitative traits was also reported in literature (Cheng, 1978; Pandey and Sinha, 1984; Sharma and Sharma, 1998; Sharma and Sharma, 2002). It was reported that *J. nigra* is known as rootstock tolerant to crown gall, *Phytophthora* and oak root fungus and induces precocity (Delbos *et al.*, 1982) and control the vigour of scion while *J. mandshurica* is cold hardy, used as a rootstock in cold area of northern China (Cheng, 1978). Its nuts are rich in protein (29%), found to be a vigorous rootstock for walnut at Shimla conditions.

Identifying correlated traits will help in the selection process in breeding programme. Table 3 reveals highly significant and positive phenotypic correlation between nut weight and its associated traits like nut width, nut length, shell weight, shell thickness and kernel weight. Such trend of correlation was also reported by Arzani *et al.* (2008). All the associations studied with respect to studied nut traits are significant and positive except those between shell thickness and nut length/kernel weight. All these traits have an impact on kernel recovery, since it is an arithmetic value arising from nut and kernel weight. Although the parameter catkin length is not studied, it appears that more the length of catkin, more will be the staminate flowers.

Nut quality depends on number of parameters notably light nut and kernel colour, smooth shell surface, shell softness, shell seal, nut and kernel weight of 12-18 and 6-10g respectively, satisfactory kernel flavour, thin or soft shell, ease in extraction of kernels from nuts and kernel recovery of more than 50 per cent (McGranahan and Leslie, 1990; Forde and McGranahan, 1996). Achieving every desired trait in single cultivar/line is very difficult if not impossible. Promising accessions identified for individual traits affecting nut quality are given below, which can serve as donors in breeding programme.

- | | |
|------------------------|--|
| 1 Nut weight (>12g) | : EC036744, EC038837, EC038836, IC019369, IC019376, IC020070, IC020107, IC020108, IC020114, IC020119 |
| 2 Smooth surfaced nuts | : EC024507, EC024562, EC026891, EC027484, EC031071, EC035719, EC036748, EC036752, IC019378, IC020109 |

3	Satisfactory kernel flavour	: Except EC036744, all have satisfactory flavour
4	Light nut colour	: EC038653, IC019378, IC019371, IC020108
5	Light kernel colour	: EC031071, IC019369, IC019378, IC020070, IC020108
6	Shell thickness (<1.5mm)	: EC024562, EC026891, EC038653, IC020108, IC558115
7	Soft shell	: EC024507, EC024562, EC026891, EC038836, EC038837, IC019371, IC019378
8	Kernel weight (>5g)	: EC038836, IC019369, IC019376, IC020108, IC538531
9	Kernel percentage (>40)	: IC538531, EC024562, EC026891, EC027484, EC036746, IC019378, IC020067, IC020108
10	Ease in extraction of kernel	: EC026860, IC019371, IC020107

Although shell thickness and soft shell has negative correlation, it may not be always so as evident from above i.e. accessions EC038653, IC020108, IC558115 had shell thickness of less than 1.5mm, still could not find place in accession with soft shell. Analysis of nut traits, productivity status, and other important traits revealed that two accessions namely IC019378 and IC020108 fulfilled many criteria as mentioned above and merit for popularization through vegetative means. Apart from many good traits, IC019378 possess desired 'round' nuts with concurrent pollen dehiscence and stigma receptivity as well.

Table 3. Phenotypic correlation coefficients for important nut traits in 32 walnut accessions studied

	Nut length	Nut width	Nut weight	Shell weight	Shell thickness
Nut width	0.80**				
Nut weight	0.73**	0.89**			
Shell weight	0.64**	0.84**	0.96**		
Shell thickness	0.06	0.37*	0.47**	0.64**	
Kernel weight	0.76**	0.80**	0.85**	0.67**	0.08

**with 1% level of significance; *with 5% level of significance

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Variability and Diversity Studies in Rain-fed Rice (*Oryza sativa* L.)

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Forty four indica rice cultivars were evaluated under rain-fed condition for sixteen quantitative traits to examine the nature and magnitude of variability and genetic divergence. Among all the traits grain yield per plant exhibited highest estimate of PCV (29.11) and GCV (28.48) followed by filled grains/panicle and effective tillers/plant. Broad sense heritability (98.90) was highest for chlorophyll content followed by grain length and grain breadth. On the basis of D^2 analysis 44 rice genotypes were grouped into nine clusters and three ungrouped clusters. Cluster I was the largest and contained 22 genotypes. Rest of the clusters contained 2-3 genotypes. The genotype belonging to ungrouped cluster X, namely, NDR 1130-1 showed promise for early maturity, long grain size and grain yield/plant. The highest intra-cluster distance was observed in cluster IX, containing two genotypes differing mainly for maturity and grain yield/plant. The most distant clusters were Cluster IV and ungrouped cluster XII. The genotypes of cluster IV showed promise for rain-fed condition with regard to days to maturity, effective tillers/plant, grain yield/plant and chlorophyll content. Contribution (%) towards the total divergence was maximum through grain breadth followed by chlorophyll content, grain length and total tillers/plant.

Key Words: D^2 analysis, Variability, Heritability, Rice, Clusters

Introduction

Rice (*Oryza sativa* L.) is the most important cereal food crop of the world and about 90 per cent of the people of South-East Asia consume rice as staple food. According to FAO, the productivity level of rice in India is very low (3.21 t/ha) as compared to the average productivity of the China (6.35 t/ha) and world (4.15 t/ha) (Anonymous, 2008). Further, a majority of area under rain-fed rice cultivation suffer from poor productivity due to lack of promising rice cultivars under such eco-climatic condition. Thus, in order to improve the productivity level, a breakthrough would be desirable by way of increasing biological efficiency through hybridization and the productivity of new varieties suited to rain-fed condition. Perhaps there is no other single crop possessing as enormous variation as in rice. However, due to the ever-increasing demand of food grains, there is still a great scope to develop rice varieties suited to rain-fed conditions for higher production. The success of this to a large extent would depend on the exploitation of existing variability and therefore it is desirable to collect, evaluate and utilize the available diversity to suit specific need with regards to specific ecosystem. Study of variability parameters and D^2 -statistics are expected to provide reliable basis for selecting out desirable elite and diverse parents for hybridization and exploitation of variability. Grouping of genotypes on the basis of D distance finally provides a

clear picture about the inter-relationship of the genotypes and helps to pick up appropriate genotype.

Materials and Methods

The experimental material comprised of 44 rain-fed rice genotypes obtained from Directorate of Rice Research Institute, Hyderabad. These genotypes were evaluated in Completely Randomized Block Design with three replications during *Kharif* 2008-2009 at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The crop was directly seeded under the rain-fed condition on 16th June, 2008. Each plot consisted of three rows of 2.25 m length. Spacing was maintained at 15 × 10 cm excluding the artificial irrigation; the normal recommended agronomic practices were followed. Fertilizers were applied at the rate of 60 Kg N₂, 40 Kg P₂O₅ and 30 Kg K₂O per hectare. Ten plants from the middle row of each entry in each replication were randomly taken for recording observations on grain yield and 15 yield attributing traits namely, days to first panicle initiation (DI), days to 50% flowering (DF), Days to 75% maturity (DM), total tillers plant⁻¹ (TN), total effective tillers plant⁻¹ (TE), plant height (PH), length of flag leaf (LF), breadth of flag leaf (BF), panicle length (PL), filled grains panicle⁻¹ (FG), fertility per cent (F%), grain weight (GW), grain length (GL), grain breadth (GB), grain yield plant⁻¹ (GY) and chlorophyll content (CL). Plot means were used for statistical analyses. Analysis of variance and variability parameter were estimated following Panse and Sukhatme (1985).

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Mahalanobis (1936) D^2 -statistics was employed to measure the genetic distance between genotypes. The genotypes were grouped in to possible number of clusters by Tocher's method as described by Rao (1952).

Results and Discussion

(A) Analysis of Variance, Range, Mean, Variability, Heritability, Genetic Advance, Phenotypic and Genotypic Coefficient of Variation

The analysis of variance for 16 traits including grain yield and its related traits in the present set of rice genotypes revealed significant differences for most of the traits. This suggested that there is an inherent genetic difference among the genotypes. Similar finding for various traits in the rice genotypes were also reported by many rice workers (Akter *et al.*, 2004 and Singh *et al.*, 2007). The estimates of range, mean, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (broad sense) and genetic advance are presented in Table 1. Considerable range of variation was observed for all the traits under study indicating enough scope for bringing about improvement in the desirable direction. Some genotypes showed promise for more than one character viz., RAU 3036 recorded it for fertility per cent, grain yield per plant, and chlorophyll content. Rewa 691 IR 78908-1015 had maximum grain yield per plant followed by Rewa 673-RR 418. Both PCV and GCV estimates were highest for grain yield per plant (29.11/28.48) followed by filled grain per panicle (25.87/24.71) and total number of effective tillers per plant (24.57/24.37). Lowest magnitude of PCV was recorded for grain length (8.03) followed by panicle length (8.31). The differences between PCV and GCV were very small for most the traits indicating lesser contribution of

environmental variation towards expression of these traits. Similar observations were also recorded by Karad and Pol (2008) in rice genotypes. The Broad sense heritability was highest for the chlorophyll content (98.9%) followed by both grain length and grain breadth (98.7%). Madhavilatha *et al.* (2005) studying with 54 rice genotypes reported high broad sense heritability for kernel L/B ratio. Genetic advance as percent of mean was, highest for grain yield per plant (57.40) followed by total number of effective tillers per plant (49.79), filled grain per panicle, effective tillers and grain weight. High heritability coupled with high genetic advance as percent of mean in the in the present set of rice genotypes was recorded for total number of tillers per plant, total number of effective tillers per plant, grain yield per plant and filled grain per panicle indicating predominance of additive gene effects and the possibilities of effective selection for the improvement of these traits. This is in consonance with the reports of Singh *et al.* (2007) in a variability study of 117 diverse rice lines. The characters like panicle length and grain length recorded high heritability but poor genetic advance which may be due to comparatively more non-additive gene effects. Hence selection would not be much effective for such characters. Similar report was also made by Satyanarayana *et al.* (2005).

(B) Genetic Divergence

χ^2 test applied to 'V' statistic was significant, indicating significant difference between the means in respect of pooled effect of 16 traits under study and between different populations. Forty four genotypes in the present study could be grouped into nine distinct clusters and three ungrouped clusters on the basis of Tocher's method of clustering utilizing D^2 values. Clustering pattern indicated that 22 out of 44 genotypes belonged to a single cluster

Table 1. Variability parameters for 16 traits in 44 genotypes of rice

Parameter		Traits															
		DI	DF	DM	TN	TE	PH	LF	BF	PL	FG	F%	GW	GL	GB	GY	CL
Range	Min.	63.00	66.33	88.67	7.00	6.10	75.13	23.61	0.96	20.59	64.33	50.16	9.93	5.83	1.50	6.60	23.03
	Max.	105.00	109.67	133.00	20.10	18.40	158.67	53.59	1.85	29.30	168.33	94.67	27.20	8.10	2.97	34.54	44.73
Mean		78.80	82.06	103.47	10.71	9.50	103.54	34.56	1.30	25.43	104.01	74.28	19.87	6.90	2.17	19.25	35.35
SEm (±)		1.317	1.395	1.197	0.244	0.238	2.254	1.468	0.039	0.653	6.491	4.019	0.953	0.051	0.025	0.950	0.359
PCV (%)		14.20	13.72	11.39	23.81	24.57	16.95	17.69	14.23	8.31	25.87	14.69	19.05	8.03	12.60	29.11	11.64
GCV (%)		14.06	13.57	11.30	23.65	24.37	16.74	16.90	13.74	7.69	24.71	13.12	18.13	7.98	12.52	28.48	11.57
Heritability (%)			97.9	97.7	98.5	98.6	98.4	97.5	91.4	93.3	85.7	91.3	79.7	90.5	98.7	98.7	95.7
98.9																	
Genetic advance (K= 2.06)		22.58	22.67	23.91	5.18	4.73	35.25	11.50	0.36	3.73	50.59	17.91	7.06	1.13	0.56	11.05	8.38
Genetic advance (% of mean)		28.65	27.63	23.11	48.37	49.79	34.04	33.28	27.69	14.67	48.64	24.11	35.53	16.38	25.80	57.40	23.71

i.e. cluster I. On the other hand, cluster II, III and IV comprised three genotypes each and cluster V, VI, VII, VIII and IX comprised two genotypes each. Three of the forty four genotypes namely NDR 1130-1, NDR 8588 (IR77770-115-MFT-B-47-6) and RAU 3036 were ungrouped and belonged to ungrouped clusters, X, XI and XII, respectively. Genotype NDR 1130-1 had highest 1000-grain weight and the longest grain length was observed for the genotype belonging to ungrouped cluster XI followed by the genotype of ungrouped cluster X (Table 3). Intra and inter-cluster distances ($\sqrt{D^2}$) among nine clusters and three ungrouped clusters have been presented in Table 2. Highest intra-cluster distance was observed for cluster IX (30.23) which comprised two genotypes differing mainly for plant height, flag leaf length, filled grains per panicle and grain yield per plant. The genotype Rewa 691 IR 78908-1015 belonging to this cluster on the basis of *per se* performance was recorded as highest yielder with early maturity and thus showed promise for rain-fed condition. The highest inter-cluster

distance (67.56) was found between cluster IV and ungrouped cluster XII followed by inter-cluster distance between cluster IV and cluster V (59.04) and between cluster V and VII (58.50). The genotypes of the most distant cluster and ungrouped cluster i.e. cluster IV and XII were quite contrasting in performance with respect to grain yield per plant and chlorophyll content. The genotypes of cluster IV showed promise with regard days to maturity, effective tillers number, grain yield per plant & chlorophyll content (Table 3). The contrasting genotypes of these two clusters are expected to yield desirable segregants on hybridization following single plant selection in later generations, suitable for rain-fed condition with early maturity and high yield potential. The smallest inter cluster distance (28.17) was observed between cluster III and ungrouped cluster X which differed mainly for the characters grain length and grain yield per plant. The small inter cluster distance indicates less diversity between the genotypes contained in these clusters. However, these genotypes can be undertaken for hybridization in order

Table 2. Average Intra and Inter-cluster “D²-values among twelve clusters of 44 rice genotypes

I. Clusters											
25.19	35.68	34.56	39.72	42.82	36.12	32.42	29.97	30.90	32.02	29.92	44.03
28.39	36.07	52.63	44.89	34.38	35.82	40.67	42.14	40.76	37.02	47.20	
26.39	47.47	41.42	44.49	43.92	37.02	34.90	28.17	39.56	57.33		
26.67	59.04	57.94	42.96	45.69	37.83	40.88	51.24	67.56			
28.31	46.32	58.50	53.97	47.15	35.91	45.60	43.38				
14.83	37.96	36.05	39.07	51.17	44.17	41.24					
25.70	30.52	39.81	47.35	37.45	55.05						
24.36	32.21	44.37	37.75	54.92							
30.23	36.12	45.10	54.53								
0.00	31.85	50.13									
0.00	36.85										
0.00											

Cluster numbers X to XII are assigned to ungrouped genotypes

Table 3. Cluster mean values for different traits

Clusters	DI	DF	DM	TN	TE	PH	LF	BF	PL	FG	F%	GW	GL	GB	GY	CL
	76.61	79.78	101.42	10.66	9.54	103.48	33.81	1.28	25.92	100.44	74.04	20.36	6.96	2.11	19.15	34.85
	99.67	103.89	125.89	9.48	8.26	89.47	35.47	1.36	23.73	105.73	69.44	19.27	7.04	2.27	16.73	37.46
	67.89	71.56	93.67	9.46	7.93	108.01	38.59	1.48	25.285	113.16	75.08	21.73	6.96	2.66	19.82	38.57
	70.00	73.44	93.22	17.83	16.00	96.00	30.54	1.17	23.22	76.18	78.54	21.27	6.81	2.17	25.91	39.19
	86.67	89.17	107.17	8.98	7.55	156.67	43.47	1.61	26.00	118.70	77.88	22.80	7.13	2.72	20.45	29.05
	94.50	97.83	119.67	8.00	7.70	98.27	41.50	1.58	24.83	161.20	64.46	12.80	5.92	2.00	16.28	31.95
	90.33	92.50	115.17	11.48	10.08	89.80	30.07	1.22	27.41	114.83	63.55	15.90	6.92	1.75	18.50	41.35
	68.33	70.50	90.33	9.30	7.90	97.42	34.07	1.16	22.39	85.27	92.07	19.30	6.35	2.00	13.79	39.15
	67.17	70.50	96.17	11.80	10.83	102.90	30.48	1.32	24.93	117.10	88.23	21.40	5.98	2.32	27.30	34.13
	67.67	74.00	93.33	11.30	9.40	112.57	35.01	1.41	26.58	118.53	77.14	23.40	7.77	2.50	26.25	33.60
	84.67	86.67	104.33	8.00	7.20	90.30	36.03	0.97	26.93	93.93	70.36	18.73	8.10	2.00	13.05	34.90
	102.67	107.33	128.33	7.900	6.90	105.87	32.57	1.05	27.43	74.93	50.16	12.93	7.43	2.00	6.60	23.03
% (**)	6.34	0.00	3.80	15.33	0.32	5.40	0.32	0.53	0.32	0.21	0.00	0.74	19.98	23.04	1.70	21.99

DI = Days to first panicle initiation, DF = Days to 50% flowering, DM = Days to 75% maturity, TN = Total tillers plant⁻¹, TE = Total effective tillers plant⁻¹, PH = Plant height (cm), LF = Length of flag leaf (cm), BF = Breadth of flag leaf (cm), PL = Panicle length (cm), FG = Filled grains panicle⁻¹, F% = Fertility %, GW = Grain weight (g), GL = Grain length (mm), GB = Grain breadth (mm), GY = Grain yield plant⁻¹ (g), and CL = Chlorophyll content. ** = Contribution of traits towards divergence.

to exploit variability for the specific characters for which the genotypes of the two clusters showed marked difference. The genotypes contained in cluster V, VIII and ungrouped cluster XII seem to be quite promising for many of the yield attributing traits under study such as, flag leaf length, flag leaf breadth, grain weight, chlorophyll content etc. Percentage contribution of the individual character towards divergence was maximum through grain breadth (23.04 %) followed by chlorophyll content (21.99%), grain length (19.98) and total number of tillers per plant (15.33%). More than 75 per cent contribution towards total divergence was mainly because of these four characters. Kole (2000) reported that the characters grain length, panicle number, grain weight and days to flowering were the major contributing traits to total diversity in a set of 20 Aromatic rice genotypes. It is observed that the genotypes desirable for different traits belonged to different clusters. The selection of diverse genotypes with desirable traits and interturn utilizing them for multiple crossing programme amongst themselves is expected to be effective in accumulation of favorable genes for bringing together different desirable traits in to the common genetic background. Thus, an improved genotype suitable for rain-fed ecosystem can be developed by utilizing such diverse genotypes in rice.

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***Vigna dalzelliana* (O. Kuntz) Verdc.: A New Distributional Record from Andaman Islands, India**

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The present paper deals with a new distributional record of *Vigna dalzelliana* from Andaman Islands. Growing side by side with *Vigna pilosa*, this species is entirely different from the hitherto reported *Vigna* species from Andaman Islands. As compared to the mainland specimens of *V. dalzelliana*, it is more robust and has deep yellow flower colour and high seed mottling.

Key Words: *Vigna dalzelliana*, Distribution, Andaman Islands

Vigna Savi, a genus of about 150-200 species distributed in tropical Africa and Asia is represented by 7 species in Andaman Islands. They are *Vigna adenantha* (G. Mey.) Marechal, *Mascherpa* and Stanier, *V. luteola* (Jacq.) Benth., *V. marina* (Burm.f.) Merr, *V. pilosa* (Willd.) Baker and the cultivated taxa *V. unguiculata* (L.) Walp., *V. mungo* (L.) Hepper and *V. trilobata* (L.) Verdc. Of these *V. marina* occurs in Great Nicobar Islands also (Hajra and Rao, 1999). While collecting crop genetic resources from Andaman and Nicobar Islands (A&N Islands), the first author has come across a strikingly different entity which upon detailed examination at Botanical Survey of India, Port Blair (PBL) was found to be not represented in PBL. The specimen was collected in 2006 as seed and herbarium (IC541388; JS/06-22) from Janakpur, Rangath, Middle Andaman, A&N Islands at 12.508°N, 92.908°E and 19 msl (Fig. 2). Herbarium specimens were compared with labeled specimens at MH and identified as *V. dalzelliana* (O. Kuntz.) Verdc. Specimens were deposited at PBL and National Herbarium of Cultivated Plants (NHCP). Seeds were regenerated at National Bureau of Plant Genetic Resources (NBPGR) Regional Station, Thrissur, Kerala, India during June to December 2007 and detailed morphological observations were made. Multiplied seeds were deposited for long-term storage at National Gene Bank, NBPGR, New Delhi (Fig. 3). In a subsequent exploration to the Andaman Islands in November 2008, three more collections were assembled (IC567253:JAS-08-29; IC567255:JAS-08-31 and IC567264:JAS-08-40).

Natural Distribution: It is reported to occur in Indian sub-continent, Thailand, Cambodia, Vietnam and Philippines. In India it is reported to be occurring widespread in eastern and southern India in Bihar, Eastern

Uttar Pradesh, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa and Rajasthan (Sanjappa, 1992). It is not reported in the Flora of Java by Baker and Brink (1963).

Description of the Species as per Cooke (1992):

Herbaceous, twining or creeping, rooting at nodes (in the absence of support), stems filiform, striate, glabrous. Leaves trifoliate, petioles ½ to 1 ½ in., ovate or rhomboid-ovate with a tendency to become lobate, acuminate, more or less hairy on both surfaces, green above, pale beneath, petiolules 1/16 in. long, stipule minute lanceolate. Flowers in capitate, 2-3 flowered racemes, peduncle filiform, 1-2 in. long, pedicels short, bracteoles 1/5 in. long, linear-subulate. Calyx 1/10 in. long, glabrous, teeth shorter than the tube, the 2 upper connate except at the tip, the two lateral triangular, obtuse, the lowest longer, lanceolate. Corolla 1/5 in. long, yellow. Pods 1¼ to 2¼ in. long, sub-cylindric, slightly curved, beaked, quite glabrous. Seeds 8-10, sub-cylindric, truncate, smooth, dark brown.

Specific Observations on Specimen of IC541388 (Fig. 1)

Seed germination hypogeal, hypocotyl colour greenish purple, primary leaves petiolate with cordate shape; annual climbing herb, stem tender, greenish purple with thread like white hairs; leaves trifoliolate, sparsely pubescent, petiole and petiolule green, terminal petiolule length medium (1.0 cm), leaflets thin, ovate, apex acute, not lobed, sparsely pubescent with white short hairs less than 0.5 mm, green with no white patches; stipules peltate, narrowly elliptic, minute, ciliate; inflorescence, auxiliary, flowers many, dense, racemes above canopy; peduncle greenish purple, sparsely pubescent, long (12.1-20.0 cm); flower-bracteole size minute, lanceolate, shorter than



Fig. 1: Plant type of specimen collected from Andaman

calyx, ciliate; calyx purplish green, flower bud small, flower color golden yellow, corolla yellow, keel curving through more than 260 degrees, pocket length medium; pollen exine with coarse reticulation. Style curving through more than 260 degrees; legume-pods glabrescent, slightly curved, length long (5.1-8.0 cm) with pointed beak, pod cross section semi-flat, pod attachment to peduncle is

pendent, no. of pods per peduncle 2-3, immature pod colour intermediate green, mature pod colour tan. Seed - 8-14 per pod, oblong, size 3.26x1.58mm, greyish green mottled with black, lustrous mottling on seed surface heavy, hilum convex.

It varies from the iso-climatic Kerala (IC539806) and Karnataka (IC539776) in its overall robustness, prolific bearing, long duration, dark yellow flower colour, bigger flower size and intense seed mottling as depicted in Table 1. By virtue of its robustness it has potential as legume green fodder and cover crop. Since its floor coverage is high, ecological function as soil erosion control agent is worth investigating. Repeated collections from fairly distant localities adjoining forest habitat indicates its natural distribution rather than introduction and spread.

Acknowledgements

The authors are grateful to Dr. SK Sharma, Director, NBPGR, New Delhi, for the facilities and encouragement provided. Joint Directors of Botanical Survey of India, Port Blair (PBL) and Coimbatore (MH) were kind enough to permit herbarium study of *Vigna* sheets.

Table 1. Comparative growth performance of representative collections

Characters	IC541388	IC539806	IC539776
Collection locality*	Middle Andaman, A&N Islands	Poomala, Thrissur, Kerala	Athur R.G.N. Park, Hassan, Karnataka
Collection habitat	Growing wild side by side with <i>Vigna pilosa</i> in deep soil on ridges of fresh water streams	Growing wild along road sides in laterite soil	Growing wild along the paddy field bunds
Leafiness	Abundant	Intermediate	Intermediate
Leaf length (cm)	7.70	4.37	8.50
Leaf width (cm)	5.97	3.87	6.30
Petiole length (cm)	9.83	9.17	8.50
Flower size (mm ²)	1.92	1.42	1.37
No. of branches	4	2	2
Days to 50% flowering	144	132	136
Flower colour	Yellow	Pale yellow	Pale yellow
Days to first pod maturity	171	132	136
No. of pods/plant	682	310	303
Pod length (cm)	5.10	4.76	3.16
No. of seeds/pod	9.00	8.00	4.20
Seed yield/plant (g)	48.16	18.12	39.34
100 seed weight (g)	1.29	0.98	1.01
Seed mottling intensity**	Dense	Sparse	Sparse
Days to senescence	171	156	155

* location depicted in Fig 1; ** depicted in Plate 2

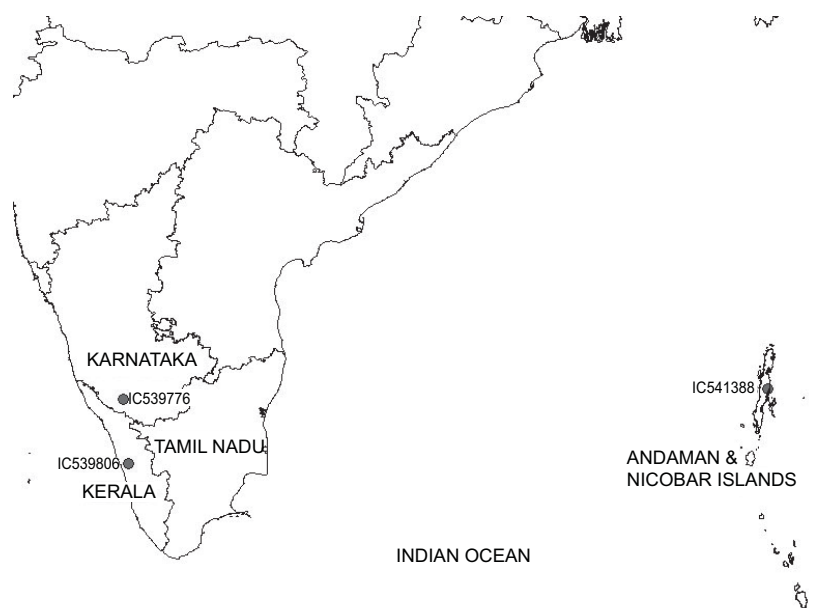
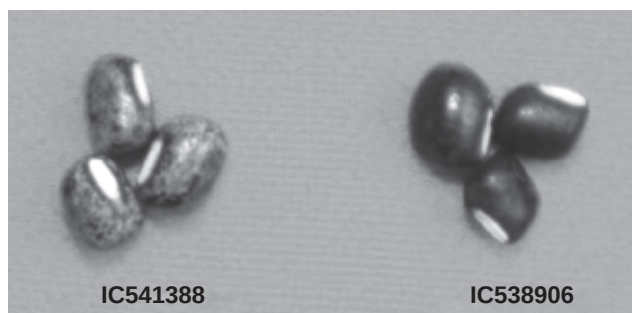


Fig. 2: Map of collection sites

Fig. 3: Seed variability in *Vigna dalzelliana*

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Genetic Diversity in Traditional Aromatic Rices of India

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Fifty-two traditional aromatic rice genotypes of India were evaluated along with three checks for quantitative characters to study genetic diversity employing D^2 analysis. Based on D^2 analysis with respect to grain yield and 14 other related traits, genotypes were classified in to four clusters and four ungrouped genotypes. Genotypes, namely, P 1174-91-4-1-1-1, Lalmati, HUR-BL-6 AR and KLS 27 belonged to four ungrouped clusters V to VIII. Genotype allocated to ungrouped cluster V was earliest for days to 50% flowering and exhibited highest mean performance fertile grain and spikelets per panicle. The genotype KLS 27 (ungrouped cluster VIII) was best for high density grain, grain length and L/B ratio. The maximum inter-cluster distance was observed between ungrouped clusters VI and VII followed by between clusters III and VIII. Tilak Chandan was promising genotype for high grain weight; Ganga Barud was local cultivar for grain yield per plant (cluster I). The genotypes having higher mean performance for various traits and diversity exhibited by their allocation in distant cluster could be considered for their inclusion in hybridization programmes to obtain desirable segregants.

Key Words: Rice, Aromatic, Genetic diversity, D^2 analysis

Introduction

Rice (*Oryza sativa* L.) is the world's single most important food crop and a primary food source for more than a third of world's population (Singh and Singh, 2008). India which is endowed with a great diversity of rice germplasm in its vast territorial land area is also known for a large number of cultivated varietal types. Amongst them, a variety of special quality rices are of great significance which deserve better premium in the domestic as well export market. So far, only basmati and long grain non-basmati varieties of rice have succeeded in the export market. Recently, after realizing the export potentiality and also the domestic demand of basmati varieties, more attention has been given to increase the yield of basmati varieties in the country. India is, however, endowed with a variety of short, slender aromatic rice varieties which are popular in different traditional rice growing pockets and commend premium no less than the traditional basmati rice. Bringing such varieties to the knowledge of consumers abroad would certainly find small but assured market for them (Siddiq, 2002). However, meager attempts have been made for the improvement of such rice genotypes. Therefore, it is very much essential to assess the extent of genetic diversity present in the aromatic rice lines of our country in order to undertake further crop improvement programmes. In the present investigation, an attempt has been made to study the nature of diversity using Mahalanobis D^2 statistic in set of traditional aromatic rice germplasm obtained from different parts of India.

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Materials and Methods

The material for the present investigation consisted of 52 traditional aromatic rice varieties/lines procured from the Directorate of Rice Research Institute, Hyderabad, India. They included the entries of Aromatic Fine Grained Observational Nursery of the All India Coordinated Rice Improvement Programme (AICRIP), India along with three checks viz., Pusa Basmati I, Mahisugandha and Type 3 (Local check). The experiment was conducted at the Agricultural Research Farm of Institute of Agricultural Sciences Banaras Hindu University, Varanasi, which represents eastern region of Uttar Pradesh in India. This site is situated at 25.2°N latitude and 83°E longitude and an altitude of 75.6 msl. The experiment was laid out in a Randomized Block Design with the two replications. Each plot consisted of six rows of two meters length. Row to row and plant to plant spacing was maintained at 20 x 15 cm. All the recommended agronomic practices were followed to raise a good crop. Observations were recorded for grain yield and 14 other traits. From each plot ten random plants were tagged for taking observations. D^2 analysis was done followed by Mahalanobis (1936). Clustering of genotypes was done by Tocher's method as detailed by Rao (1952).

Results and Discussion

In the present study, V (stat.) calculated for D^2 analysis and tested by the significance of χ^2 , revealed significant difference between the means in respect of the pooled effect of 15 characters among 52 aromatic rice genotypes. Clustering pattern along with inter- and intra- cluster

Table 1. Average inter and intra-cluster D values among eight clusters of 52 aromatic rice genotypes

Cluster	I	II	III	IV	V	VI	VII	VIII
I	48.37	81.40	111.25	106.51	93.58	99.23	118.32	115.56
II		39.78	65.61	78.87	102.41	100.45	113.40	104.32
III			36.56	80.53	90.18	95.61	106.24	120.68
IV				32.16	85.67	88.74	96.35	110.40
V					0.00	50.45	119.50	113.63
VI						0.00	125.24	102.34
VII							0.00	85.24
VIII								0.00

Cluster numbers V to VIII are assigned to ungrouped genotypes

distances have been presented in Table 1. The 52 genotypes could be divided into four clusters (Table 2). Cluster I was the largest with 34 genotypes and maximum intra-cluster distance (48.37), indicating diversity within the group but not to the extent so that they can form separate clusters as there existed genetic similarities among themselves on the basis of multiple characters causing them to belong into a single cluster. Cluster II consisted of four genotypes, while cluster III and IV contained five genotypes each. The ungrouped clusters represented by numbers V, VI, VII and VIII were assigned to four single ungrouped genotypes, namely, P 1174-91-4-1-1-1, Lalmati, HUR-BL-6 AR and KLS 27. The maximum distance (125.24) was observed between the ungrouped genotypes Lalmati and HUR-BL-6AR with contrasting mean performances for plant height, panicle weight, grain length, high density grain index, and grain yield per plant. Similarly, the ungrouped genotype KLS 27 showed the next highest inter-cluster distance (120.68) with cluster III, and exhibited higher mean performance for plant height and high density grain index compared to the mean performance of genotypes contained in cluster III (Table 2).

Among the genotypes that could be grouped into four different clusters the maximum inter-cluster distance (111.25) was observed between cluster I and III which exhibited contrasting mean performances for the characters namely, filled grain per panicle and spikelets per panicle. The next higher inter-cluster distance (106.51) was observed between cluster I and IV were as, the smallest inter cluster distance (65.61) was observed between cluster II and III. The cultivar Punjab Basmati, a well known genotype, belonged to cluster III and exhibited difference in performance with the Kariga Javile (cluster II) *per se* in respect of characters, namely, grain maturity duration, plant height, spikelets per panicle and L/B ratio. Both of these have been a collection from Amritsar district of Punjab.

In the present study, hybridization between genotypes having higher inter D-distances are expected to generate exploitable variability for the improvement in the yield and yield related traits as well as are likely to through desirable transgressive segregants in later generations of hybridization. D² analysis is an efficient statistical tool to assess the genetic diversity and has been employed by many workers in various crops, such as Brassica (Murty and Quarry, 1996), cotton (Singh and Bains, 1988), linseed (Anand and Murthy, 1968) rice (Shiva Kumar *et al.*, 1989; Subramaniam and Vivekanandan, 1993; (Sarma and Richharia, 1995 and Sarma *et al.*, 1996, 1997). In the present study, D² analysis revealed presence of considerable diversity among the genotypes investigated and the genotypes were observed to be distributed into four different clusters. Since, the genotypes present in the same cluster indicate their close relationship as compared to others (Table 1), it could be expected that all the 34 genotypes present in the cluster I were some how genetically related among themselves and were diverse from the genotypes belonging to others classes. It was interesting to note that the aromatic fine-grained check varieties included in the experiment also belonged to this cluster. This might be due to similar selection pressure applied for this group of genotypes in favour of grain quality attributes. The genotypes that were found in this cluster are the indigenous aromatic fine-grained rice varieties of different locations of our country, which generally command premium prices in the local markets.

The two selected lines from Karnal Local, *i.e.*, KLS-5 and KLS-27 were observed to belong to cluster II and ungrouped cluster VIII. This indicates the effectiveness of selections in the local land races in generating genetic variability for utilization in the crop improvement programme. The clustering pattern indicated wide diversity between different groups of genotypes. The greater the distance between two clusters, wider is the expected

Table 2. Cluster mean values for fifteen traits of 52 aromatic rices

Cluster	Traits														
	DF	DM	GMD	PH	TN	PL	PW	FG	SP	GW	GL	GB	L/B	HDI	GY
I	112.67	145.64	28.31	117.81	11.07	23.57	2.28	145.98	160.39	1.63	7.07	2.54	2.79	20.71	10.78
II	112.25	145.73	33.00	81.88	8.00	23.83	2.05	95.18	111.70	1.95	9.93	2.79	3.43	19.97	9.90
III	104.72	132.50	27.42	80.20	8.76	22.09	1.82	81.06	93.82	1.78	10.12	2.52	4.14	27.72	8.36
IV	118.90	140.46	29.48	83.60	8.88	21.18	1.96	111.15	126.31	2.22	8.48	2.97	2.97	20.27	8.59
V	95.10	123.70	28.60	84.10	7.50	21.70	2.38	163.40	180.95	1.50	8.80	2.41	3.60	24.51	8.18
VI	100.30	130.10	29.80	79.30	8.80	21.38	1.52	95.35	114.75	1.35	6.61	2.46	2.68	34.90	4.96
VII	102.00	136.20	34.70	127.50	8.60	24.85	2.61	112.75	125.45	2.35	10.46	2.68	3.84	14.09	8.49
VIII	107.20	134.20	27.10	121.80	9.70	23.38	1.91	89.80	112.00	1.35	10.69	2.64	4.06	56.73	8.42

Abbreviations:— DF = Days to 50% flowering, DM = Days to maturity, GMD = Grain maturity duration, PH = Plant height, TN = Effective tiller number⁻¹, PL = Panicle length, PW = Panicle weight, FG = Filled grains panicle⁻¹, SP = Spikelets panicle⁻¹, GW = Grain weight, GL = Grain length, GB = Grain breadth, L/B = Length breadth ratio of grain, HDI = High density grain index (%), GY= Grain yield⁻¹.

genetic distance between the genotypes. This has also been suggested by aromatic rice workers such as (Kole 2000; Sharma *et al.*, 2002). The genetic distance among the parents largely governs the variability spectrum generated in the segregating generations and also heterosis in the F₁s. Therefore, identification of genetically diverse genotypes would help in selecting desirable parents for hybridization programmes. However, it has been suggested that while selecting parents for hybridization programme their yield potential should not be overlooked (Singh *et al.*, 1987).

The characters which contributed maximum towards the total diversity in the present study were; grain maturity duration (24.03%), plant height (19.94%), panicle weight (17.18%), high density grain index (14.10%), grain weight (6.22%), and grain yield per plant (5.7%). Kole (2000) studying the genetic divergence in 20 aromatic rice genotypes including mutants reported that grain length, panicle number, grain weight, and days to flowering were the measure contributors towards the total diversity.

The diverse genotypes identified (belonging to distant clusters) with desirable performances for different traits, if are utilized into multiple crossing programmes would yield promising results in bringing together different desirable genes into the common genetic background. Sarawgi and Rastogi (2001) in aromatic rice have also advocated crossing of diverse genotypes for evolving semi-dwarf, high yielding and early maturing lines with good grain quality.

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In-situ on Farm Agro-biodiversity Conservation in Mid-hills of Indian Central Himalaya

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Central Himalayan region of India is not only a mega plant biodiversity zone but also it has a very rich agro biodiversity coverage embodying landraces and wild ancestors of domesticated plants. In the wake of depleting agro biodiversity base in the region, a study was carried out to investigate the status of *in situ* conservation of these crop plants on-farm in the areas of Tarikhet and Ukhimath Blocks of Kumaon and Garhwal regions in Central Himalayan middle hills. After the pilot survey was conducted to finalize the objective of selecting locations in 2003, a pre-tested standardized technical questionnaire based study was carried out during 2004. The areas covered in this study are less explored and underprivileged almost in the center of the state representing the ecological, agricultural and cultural portraits of both the regions. In between the similarities and dissimilarities in the cropping pattern in both the areas, the richness and intensity of agro biodiversity conservation was comparatively more in Tarikhet as to that of Ukhimath particularly while expressing *in situ* conservation of traditional and coarse grain crops on-farm. Traditional crops and cultivars are still having favor though receiving declined interest due to invasion of HYVs (high yielding varieties), change in agricultural practices, varying lifestyles and food habits. Indian authorities should stronghold the mega-measures for conserving plant genetic wealth in the wake of globally effective and widely accepted efforts of FAO for the conservation of rare, traditional and wild plant genetic resources particularly in the under-privileged areas of the country.

Key Words: Central Himalayas, Diversity distribution, Human sustenance, *In situ* conservation, Plant genetic resources

Introduction

Towards sustaining agro biodiversity and securing food for present and future generations, FAO, (the Food and Agriculture Organization of the United Nations), called upon the international community to attend a 'Plant Summit' in Leipzig in 1996 which culminated in the evolution of FAO Global Plan of Action (FAO 1999 a and b). The efforts of FAO were further strengthened when IT-PGR (The International Treaty on Plant Genetic Resources) was adopted in November 2001 which came into force on 29th June 2004 that initially concurred to manage the agro biodiversity of 35 food crops and 29 forage crops; which reflect 80% of the calorie intake of the world's population (FAO, 2001). Himalayan region stretching through almost eight countries is not only unique in its vastness among other mountain ranges in the world but also it embodies a peculiar ecological configuration, wild genetic resource base and cultural traditions. Since the region is one of the important eight gene centers of wild relatives of cultivated agro biodiversity its conservation is a vital issue (Ives and Messerli 1989; Ramakrishnan *et al.*, 1996; Buch-Hansen, 1997). Besides

the treasure of wild relatives of crop plants, a distinctive integrated approach of "Forest-Farm-livestock" in agriculture represented the Indian central Himalayan region in the past. Because of inattention at local, state and national level, the agro biodiversity of traditional crops and their wild relatives faced the threat of extinction in previous years. However, a few efforts (Nautiyal *et al.* 1998 a and b; Maikhuri *et al.*; 2001; Maikhuri *et al.*; 2002; Rao *et al.* 2003) have been made to study the prevailing trends on traditional agro biodiversity in farming systems. In view of national ecological perspective, this study was carried to document the *in situ* conservation trends of agro biodiversity in underprivileged and unexplored areas in Tarikhet and Ukhimath Blocks.

Material and Methods

Study Sites

In Uttarakhand, Tarikhet and Ukhimath blocks of Almora and Rudrapur districts fall in Kumaon and Garhwal regions respectively. It is around 94.546 ha agricultural land in Tarikhet at an altitude between 1700-1800 msl (meter above sea level) while Ukhimath is situated at an

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altitude between 1300-1500 msl and covering the cultivated area approximately 106.315 ha. Geophysical locations for Tarikhet are approximately between $79^{\circ} 22'$ to $79^{\circ} 29'$ longitude and $29^{\circ} 33'$ to $29^{\circ} 41'$ latitude as well as for Ukhimath are $79^{\circ} 09'$ to $79^{\circ} 18'$ longitude and $30^{\circ} 30'$ to $30^{\circ} 41'$ latitude (Fig. 1).

Strategy

A team of multidisciplinary researchers having specializations in anthropological studies, socio-ethnobotany, socio-economics, agro-ecology, crop production systems, crop protection, plant breeding and plant genetic resources was involved in this investigation before finalizing the pilot survey during 2003. The information on agro biodiversity management and rural development through archival records in institutions were examined to determine the locations suitable for investigation. The areas covered in this study are less explored and underprivileged almost in the center of the state representing the ecological, agricultural and cultural

portraits of both Kumaon and Garhwal regions. After the pilot survey, a pre-tested standardized technical questionnaire based study was carried out during 2003 and 2004. These questionnaires gave the common basis of information on the *in situ* conservation of agricultural biological diversity on-farm. Throughout the study, different level of sources like local people, regional experts and local institutions were involved while confirming the information before the synthesis of data.

Sample Selection Criteria

The standardized methodology for sample selection was adopted. From both the blocks, ten percent villages were selected and out of those, fifteen percent populations constituted the lots of samples. A ratio of composition of communities, castes and scheduled tribes was maintained corresponding to their representation in the area. The criteria of sample selection were completely randomized and all parameters like upland and lowland attributes, village demographic features, economic and

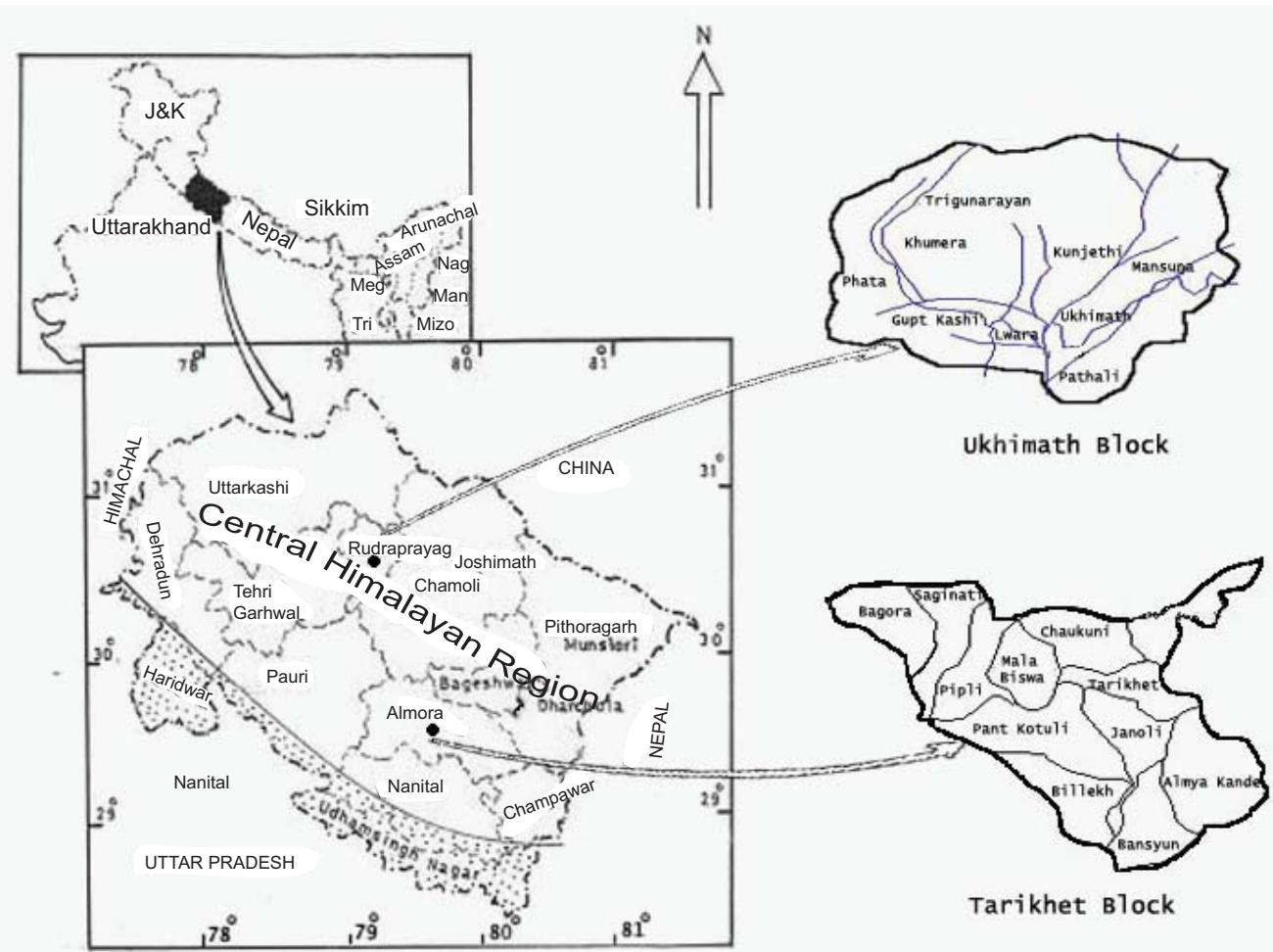


Fig. 1: Location map of the area of study

land holding levels of farmers, land types were equally ratified while recording the observation on agro biodiversity.

Results and Discussion

Depending upon the seasons (October to March-Rabi season, April to September-Kharif season), the variability in selection of agro biodiversity for cultivation in hills was comparatively a typical in comparison to plain areas in northern parts of the country apart from some base crops like wheat, rice, blackgram and some vegetables common to both areas. In middle hills, traditional crops and cultivars are still having favour though receiving declined interest due to invasion of HYVs (high yielding varieties), change in agricultural practices, varying lifestyles and food habits. The changing agricultural trends in Central Himalayan middle hills due to various factors described earlier (Ramakrishnan *et al.*, 1996, Nautiyal *et al.* 1998 a and b, Maikhuri *et al.*, 2002, Rao *et al.* 2003).

Landscape Orientation

There was not much difference in the landscape orientation in the areas studied under Tarikhet and Ukhimath Blocks except matured stony hills in former as compared to less-matured and muddy hills in Ukhimath areas. The habitat of both the locales (Tarikhet and Ukhimath) encompassed sloppy contoured agricultural fields along with the elevation sides of hills intensively mixed with valleys and also on some locations with grassland steppes amidst pine forests and in open space. The slope of lands was steeper in Tarikhet region as compared to Ukhimath lands. Micro-habitats could easily be recognized among ecosystems. Apart from pines and other moist deciduous forests diversity areas were densely coupled with the MAP (medicinal and aromatic plants) genetic resources like *Valeriana wallichii*, *Rauwolfia serpentina* etc. characterized with more frequency. At some places in Tarikhet Block, it was a scenario of dry stony hills having appearance of semi cold desert or cold desert in winter. At some locations, the mighty stony wet hills were covered with groups of lower vegetation in Tarikhet having marginal lands for scattered farming. Mostly low lands rich in humus along valleys were suited and occupied with terraced farming. The elevated mountainous locations were having distinct pattern of almost undisturbed biodiversity due to variation in climatic profile. The natural forests in Ukhimath were dominant with trees having broad leaves, a characteristic feature of Garhwal forests, afforded as a major source of nutrient supply to

soil. The traditional ecological link of forest-farm-livestock cycle was stronger and functional in Ukhimath as compared to Tarikhet.

Agro Biodiversity On-farm in Tarikhet

We documented total 91 plant species in the area Tarikhet block. Out of these, 23 plants comprise food grains, pulses and oil seeds; vegetable and cash crops aggregates to be 27 while 20 plant species belong to fodder and agro forestry group. The agro biodiversity in fruit accounts to be around 21 species (Table 1). Vegetables and cash crops share major chunk of agro biodiversity that is

Table1. In situ agro-biodiversity conservation on-farm in Tarikhet

Botanical Name of plants	English Name	Local Name	Season of Cultivation
(1)	(2)	(3)	(4)
Food crops			
<i>Amaranthus caudatus</i>	Chaulai	Ram Dana	K
<i>Cajanus cajan</i>	Pigeon pea	Arhar	K
<i>Echinochloa frumentacea</i>	Barnyard Millet	Madira	K
<i>Eleusine coracana</i>	Finger Millet	Mandua	K
<i>Glycine max</i>	Soyabean	Soyabean/ Bhat	K
<i>Macrotyloma uniflorum</i>	Horse gram	Gahat	K
<i>Oryza sativa</i>	Rice	Dhan	K
<i>Phaseolus vulgaris</i>	Kidney Bean	Rajmah	K
<i>Sesamum indicum</i>	Sesame	Til	K
<i>Setaria italica</i>	Fox Tail Millet	Koni	K
<i>Sorghum bicolor</i>	Jowar	Jowar	K
<i>Vigna mungo</i>	Blackgram	Urad	K
<i>Vigna radiate</i>	Mung	Green gram	K
<i>Vigna sinensis</i>	Cow pea	Lobiya	K
<i>Zea mays</i>	Maize	Makka	K
<i>Brassica alba</i>	White mustard	Rai (white)	R
<i>Brassica napus</i>	Mustard	Sarso	R
<i>Brassica nigra</i>	Black mustard	Rai (black)	R
<i>Cicer arietinum</i>	Gram	Chana	R
<i>Hordeum vulgare</i>	Barley	Jau	R
<i>Lens culinaris</i>	Lentil	Masoor	R
<i>Triticum aestivum</i>	Wheat	Gehu	R
Vegetables and cash crops			
<i>Colocasia esculenta</i>	Taro	Arvi	K
<i>Cucumis melo</i>	Muskmelon	Kharbooja	K
<i>Cucumis sativus</i>	Cucumber	Khira	K
<i>Cucurbita maxima</i>	Pumpkin	Kaddu	K
<i>Dioscorea alata</i>	Winged yam	Khamalu	K
<i>Hibiscus esculentus</i>	Okra/Lady fingers	Bhindi	K
<i>Luffa cylindrica</i>	sponge gourd	Tori	K
<i>Momordica charantia</i>	Bitter gourd	Karela	K
<i>Solanum melongena</i>	Eggplant	Baigan	K
<i>Spinacia oleracea</i>	Spinach	Palak	K
<i>Allium cepa</i>	Onion	Piyaz	R
<i>Allium sativum</i>	Ginger	Adrakh	R
<i>Brassica botrytis</i>	Cauli flower	Gobhi (phool)	R
<i>Brassica juncea</i>	Leaf mustard	Rai/Sarson	R
<i>Brassica oleracea (capitata)</i>	Cabbage	Gobhi (Patta)	R
<i>Capsicum annum</i> var. <i>annuum</i>	Capsicum	Shimalaa Mirch	R

(1)	(2)	(3)	(4)
<i>Curcuma domestica</i>	Turmeric	Haladi	R
<i>Daucus carota</i>	Carrot	Gajar	R
<i>Dolichos lablab</i>	Beans	Sem	R
<i>Lagenaria siceraria</i>	Bottle gourd	Lauki	R
<i>Pisum sativum</i>	Pea	Matar	R
<i>Paspalum scrobiculatum</i>	Kodo Millet	Kodon	R
<i>Solanum lycopersicum</i>	Tomato	Tamatar	R
<i>Solanum tuberosum</i>	Potato	Alu	R
<i>Trigonella foenum-graecum</i>	Fenugreek	Methi	R
<i>Zingiber officinale</i>	Ginger	Adrakh	R
Fodder and Agroforestry plants			
<i>Acacia nilotica</i>	Acacia	Babool	Fr
<i>Aesculus indica</i>	Horse Chest nut	Pankor	Ft
<i>Alnus nepalensis</i>	Nepalese Alder	Uti	Fr
<i>Bambusa arundinacea</i>	Bamboo	Bans	Fr
<i>Celtis australis</i> linn	European	Khari hackberry	Fr
<i>Ficus palmate</i>	Wild fig	Anijir	Fr
<i>Ficus roxburghii</i>	Elephant ear fig tree	Gular	Fr
<i>Gossypium hirsutum</i>	Cotton	Kapaas	Ft
<i>Grewia optiva</i>	Bhimal+	Bhimal	Fr
<i>Melia azedarach</i>	Margosa	Bakain	Fr
<i>Morus alba</i>	Mulbury	Shehtoot	Ft
<i>Pinus roxburghii</i>	Pine	Chir	Ft
<i>Populus androsocoggin</i>	Poplar	Poplar	Fr
<i>Prunus cerasoides</i>	Wild himalayan cherry	haya/Paddam	Fr
<i>Quercus leucotrichophora</i>	Oak	Banj	Fr
<i>Rubus ellipticus</i>	Yellow Himalayan	Lalanchu Rasperry	Fr
<i>Sapindus emarginatus</i>	Soapnut	Ritha	Ft
<i>Thuja orientalis</i>	Oriental arborvitae	Morphankhi	Ft
<i>Toona ciliata</i>	Red cedar	Tun	Ft
<i>Ficus</i> spp.	Kheena+		Fr
Fruit plants			
<i>Carica papaya</i>	Papaya	Papeeta	F
<i>Citrus aurantifolia</i>	Lime	Kagzi nibu	F
<i>Citrus limon</i>	Lemon	Bara nimbu	F
<i>Citrus reticulata</i>	Orange	Santara/Narangi	F
<i>Citrus sinensis</i>	Sweet orange	Santara	F
<i>Juglans regia</i>	Walnut	Akhrot	F
<i>Litchi chinensis</i>	Litchi	Litchi	F
<i>Malus sylvestris</i>	Apple	Seb	F
<i>Mangifera indica</i>	Mango	Aam	F
<i>Musa paradisiaca</i>	Banana	Kela	F
<i>Prunus armeniaca</i>	Apricot	Zardalu	F
<i>Prunus domestica</i>	Plum	Alu bukhara	F
<i>Prunus persica</i>	Peach	Aadoo	F
<i>Prunus tomentosa</i>	Cherry	Cherry	F
<i>Psidium guajava</i>	Guava	Amrud	F
<i>Punica granatum</i>	Pomegranate	Anar	F
<i>Pyrus communis</i>	China pear	Nashpati	F
<i>Vitis vinifera</i>	Grapes	Angoor	F

R - Rabi season of cultivation- (October to March)

K - Kharif season of cultivation- (April to September)

F - Fruit species; Fr - Fodder species; Ft - Forest tree; U - Season of cultivation could not be confirmed; + - English name could not be confirmed

followed by fruit and fodder plants in total agro biodiversity as compared to food grains, pulses and oil seeds (Fig.2). It was almost much similar incidence of agro biodiversity in both the areas investigated, the cultivation of *Cicer arietinum*, *Brassica alba*, *Vigna radiata*, *Sorghum bicolor*, *Trigonella foenum-graecum* and *Dioscorea alata* though found in Tarikhet was missing in the areas of Ukhimath Block.

The presence of variability among fodder and agro forestry plants as well as fruit tree species was also found to be variable as *Melia azedarach*, *Bambusa arundanacea*, *Pinus roxburghii*, *Prunus cerasoides*, *Aesculus indica* and *Sapindus granatum* as well as *Psidium guajava*, *Punica granatum*, *Prunus tomentosa*, *Punica* spp. and *Carica papaya* respectively were restricted in Tarikhet areas only.

Figure 4 shows that ginger (*Zingiber officinale*) and cabbage (*Brassica oleracea*) occupied the least agricultural land while wheat figured the highest followed by rice and finger millet, a traditional coarse grain crop; which has prominent place in Uttarakhand agriculture in terms of production following wheat and rice. Nevertheless, Lentil (*Lens culinaris*) occupied comparatively more agricultural land followed by *Cicer arietinum* but the frequency of beans was more.

Agro Biodiversity On-farm in Ukhimath

Figure 3 illustrates that the agro biodiversity profile in Ukhimath had much resemblance to that of Tarikhet except few variations in the frequency of crops and selection criteria of farmers. Eighty plant species were found to be domesticated (Table 2), and in this area of Garhwal region also, vegetables and cash crops comprised the key hunk followed by fodder and agro forestry crops and then fruit trees. However, the agricultural land acquired by crops grown during March to October almost equaled the area coverage by fruit trees that reflect comparatively lesser biodiversity in fruit tree species in Ukhimath as compared to Tarikhet. The endemic agriculture of some tree species like *Psidium guajava*, *Punica granatum*, *Punica* spp. (other pomegranate variety locally known as Darim) *Prunus tomentosa*, and *Carica papaya* was detected only in Tarikhet. Correspondingly, the four food crops viz. *Cicer arietinum*, *Brassica alba*, *Vigna radiata* and *Sorghum bicolor* were represented only Tarikhet areas but absent in Ukhimath. In vegetables and cash crops, *Vicia faba* and *Coriandrum sativum* were found to be cultivated only in Ukhimath areas. The location specific agro biodiversity incidence was also observed

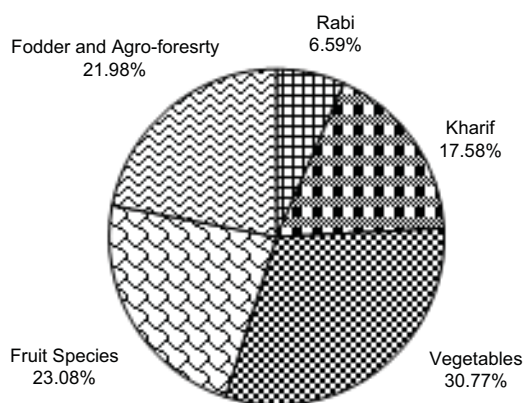


Fig. 2: Percent shares of different species in total agro biodiversity recorded in Tarikhet

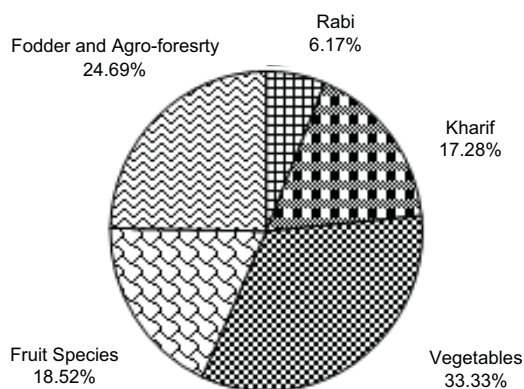


Fig. 3: Percent shares of different species in total agro biodiversity recorded in Ukhimath

Table 2. *In situ* agro biodiversity conservation on-farm in Ukhimath

Botanical name of plants	English name	Local name	Season of cultivation
(1)	(2)	(3)	(4)
Food crops			
<i>Amaranthus caudatus</i>	Chaulai	Ram dana	K
<i>Cajanus cajan</i>	Pigeon pea	Arhar	K
<i>Echinochloa frumentacea</i>	Barnyard Millet	Madira	K
<i>Eleusine coracana</i>	Finger Millet	Mandua	K
<i>Glycine max</i>	Soyabean	Soyabean /Bhat	K
<i>Macrotyloma uniflorum</i>	Horse gram	Gahat	K
<i>Oryza sativa</i>	Rice	Dhan	K
<i>Phaseolus vulgaris</i>	Kidney Bean	Rajmah	K
<i>Sesamum indicum</i>	Sesame	Til	K
<i>Setaria italica</i>	Fox Tail Millet	Koni	K
<i>Vigna radiata</i>	Mung	Green gram	K
<i>Vigna sinensis</i>	Cow pea	Lobiya	K
<i>Zea mays</i>	Maize	Makka	K
<i>Brassica napus</i>	Mustard	Sarso	R
<i>Brassica nigra</i>	Black mustard	Rai (black)	R
<i>Hordeum vulgare</i>	Barley	Jau	R
<i>Lens culinaris</i>	Lentil	Masoor	R
<i>Triticum aestivum</i>	Wheat	Gehu	R
Vegetables and cash crops			
<i>Celocasia esculenta</i>	Taro	Arvi	K
<i>Coriandrum Sativum</i>	Coriander	Dhania	K

(1)	(2)	(3)	(4)
<i>Cucumis melo</i>	Muskmelon	Kharbooja	K
<i>Cucumis sativus</i>	Cucumber	Khira	K
<i>Cucurbita maxima</i>	Pumpkin	Kaddu	K
<i>Hibiscus esculentus</i>	Okra/Lady's finger	Bhindi	K
<i>Luffa cylindrica</i>	Sponge gourd	Tori	K
<i>Momordica charantia</i>	Bitter Gourd	Karela	K
<i>Solanum melongena</i>	Brinjal	Baigan	K
<i>Spinacia oleracea</i>	Spinach	Palak	K
<i>Vicia faba</i>	Broad bean	Bakla	K
<i>Allium cepa</i>	Onion	Piyaz	R
<i>Allium sativum</i>	Garlic	Lehsun	R
<i>Brassica botrytis</i>	Cauliflower	Gobhi (Phool)	R
<i>Brassica oleracea (capitata)</i>	Cabbage	Gobhi (patta)	R
<i>Capsicum annum</i>	Capsicum	Shimla-Mirch	R
<i>var annum</i>			
<i>Capsicum annum</i>	Chilli	Mirch	R
<i>Curcuma domestica</i>	Turmeric	Haldi	R
<i>Daucus carota</i>	Carrot	Gajar	R
<i>Dolichos lablab</i>	Beans	Sem	R
<i>Lagenaria siceraria</i>	Bottle gourd	Lauki	R
<i>Pisum sativum</i>	Pea	Matar	R
<i>Raphanus sativus</i>	Radish	Muli	R
<i>Solanum lycopersicum</i>	Tomato	Tamatar	R
<i>Solanum tuberosum</i>	Potato	Alu	R
<i>Zingiber officinale</i>	Ginger	Adrakh	R

Fodder and Agroforestry Plants

<i>Celtis australis</i> linn	European hackberry	Khari	F
<i>Ficus palmata</i>	Wild fig	Anjir	F
<i>Ficus roxburghii</i>	Eve's apron	Timal	F
<i>Ficus subincisa</i>	Chanchri+	Chanchri	F
<i>Fraxinus micrantha</i>	Fraxinus	Angu/Litsia	F
<i>Fraxinus xanthoxyloides</i>	Afgahan Ash	Thelka	F
<i>Gossypium arboreum</i>	Thailand cotton	Kapaas	F
<i>Grewia optiva</i>	Not found	Bhimal	F
<i>Lyonia ovalifolia</i>	Not found	Ayar	F
<i>Morus alba</i>	Mulberry	Shehtoot	F
<i>Quercus leucotricophora</i>	Oak	Banj	F
<i>Quescus floribunda</i>	Moru Oak	Moru	F
<i>Rhododendron arboreum</i>	Rose tree	Burash	F
<i>Salix spp.</i>	willow	Karve	F
<i>Thuja orientalis</i>	Oriental arborvitae	Morphankhi	F
<i>Toona ciliata</i>	Red cedar	Tun	F

Fruit Plants

<i>Citrus auratifolia</i>	Lime	Kagzi nibu	F
<i>Citrus limon</i>	Lemon	Bara nibu	F
<i>Citrus reticulata</i>	Orange	Santara/Narangi	F
<i>Citrus sinensis</i>	Sweet orange	Malta	F
<i>Juglans regia</i>	Walnut	Akhrot	F
<i>Litchi chinensis</i>	Litchi	Litchi	F
<i>Malus sylvestris</i>	CrabApple	Seb	F
<i>Mangifera indica</i>	Mango	Aam	F
<i>Musa paradisiaca</i>	Banana	Kela	F
<i>Prunus armeniaca</i>	Apricot	Khubani	F
<i>Prunus domestica</i>	Plum	Alucha	F
<i>Prunus persica</i>	Peach	Aadoo	F
<i>Pyrus communis</i>	Pear	Naspati	F
<i>Vitis vinifera</i>	Grapes	Angoor	F

R - Rabi season of cultivation- (October to March)

K - Kharif season of cultivation- (April to September)

F - Fruit species; Fr - Fodder species; Ft - Forest tree; U - Season of cultivation could not be confirmed; + - English name could not be confirmed

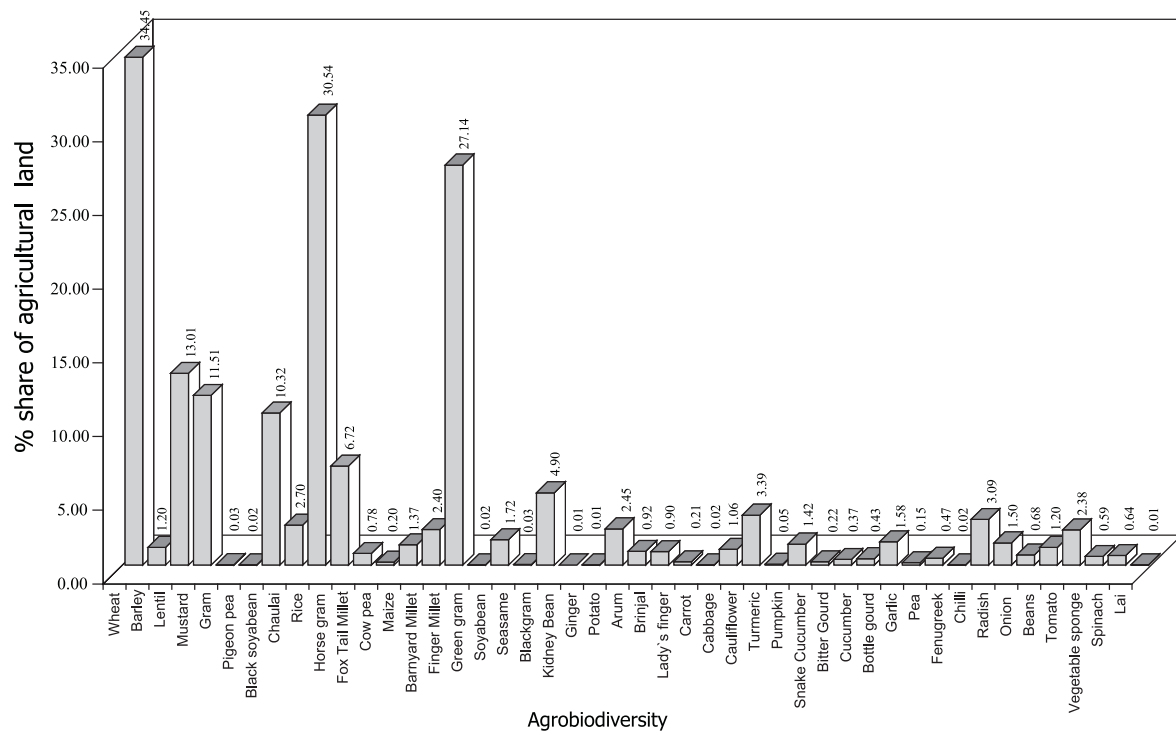


Fig. 4: Percentage incidence of agro biodiversity based on the area covered by agricultural land in Tarikhet

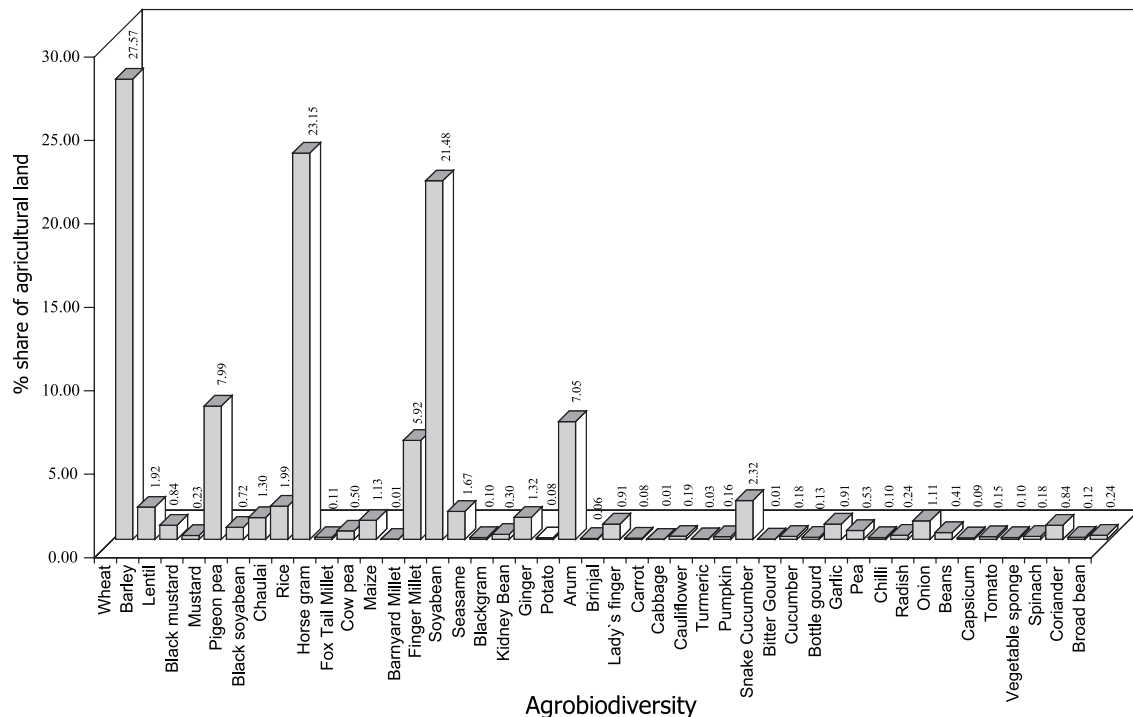
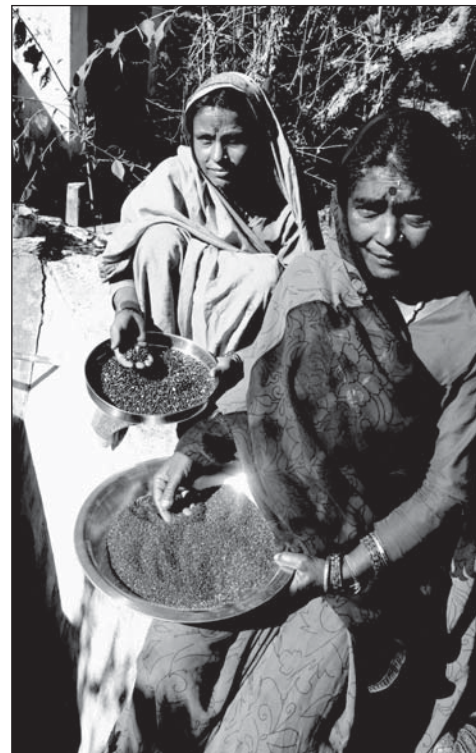
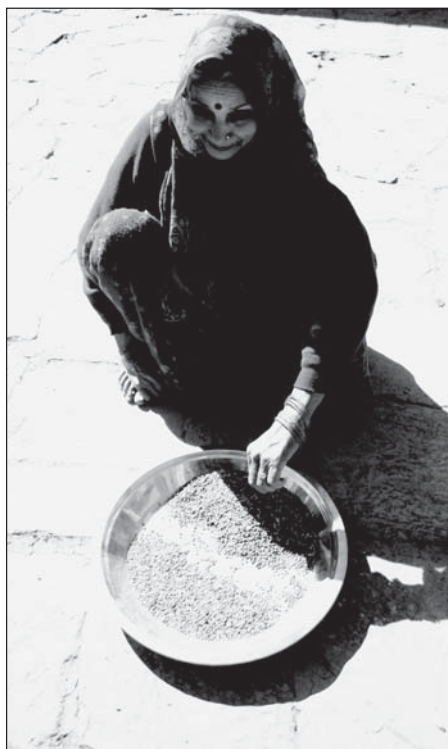


Fig. 5: Percentage incidence of agro biodiversity based on the area covered by agricultural land in Ukhimath

in fodder and agro forestry plant species in Ukhimath where the frequency of these plants was comparatively more in comparison to Tarikhet.

Among food crops, *Triticum aestivum* (wheat) ranked first in terms of cultivation followed by *Oryza sativa* (rice), *E. coracona* (finger millet) and *B. napus* (mustard).



Figs. 6 and 7: Women actively participate in the *in situ* conservation of customary crops like *Macrotyloma uniflorum*, *Eleusine coracana* and a species of *Glycine max* locally known as *Bhat*



Fig. 8: Natives in Ukhimath conserve agro biodiversity in their kitchen gardens

Conclusions

Albeit the *in situ* conservation trends of genetic resources on-farm in Central Himalayan middle hills has acquired a shift the cultivation of some traditional crops is still maintained. The persistence of crops like *Eleusine*

coracana, *Echinochloa frumentacea*, *Macrotyloma uniflorum*, *Dioscorea alata*, *Colocasia esculenta*, *Amaranthus caudatus* etc. despite depleting the customary crops in the region reflected the stakeholders' interests in these crops. And the scenario of *in-situ* conservation

of these underprivileged crops on-farm even in unsustainable agricultural systems was much better in the areas of Tarikhet Block as compared to Ukhimath. However, the cropping pattern of vegetables and cash crops was almost similar except some slight variations in both the areas. The absence of agro biodiversity of four food crops in Ukhimath may be due to several factors. A very interesting feature was perceived that the potato (*Solanum tuberosum*) farming was reasonably much significant in Ukhimath to that negligibly found in Tarikhet but this order was reverse in case of Horse gram (*Macrotyloma uniflorum*). The richness and intensity of agro biodiversity conservation was comparatively more in Tarikhet as to that of Ukhimath particularly while expressing *in situ* conservation of traditional and coarse grain crops on-farm.

So, it is a need of encouragements to crop growers from concerned departments of government, NGOs and other private sectors that deal with the biodiversity conservation and sustainable agriculture to promote cultivation of these wild relatives and landraces rich in energy and nutrition. The farmers in these areas need advancement of understanding related to the complex practices and mechanisms for on farm conservation of crop diversity and their relation with farmers' livelihoods, encouragements for profitable management practices.

In the wake of efforts made by FAO (UN) towards agro biodiversity conservation like Community Biodiversity Development and Conservation Programme (CBDC/IDRC) and Biodiversity Utilization and Conservation in Asia and the Pacific (BUCAP/NORAGRIC/ MARD) that has been well accredited globally and measures have been strengthened in various parts of the continents stretching across south-east Asia, south Asia to African continent and Latin America. Indian authorities should stronghold the mega-measures for the conservation of rare, traditional and wild genetic resources at the government's sound working policy levels, besides the foreign assistance like pilot project on home gardens to *in-situ* conservation of PGR of CIC Italy, particularly for sensitive agricultural ecosystems in Central Himalayan region.

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Development of Plant Genetic Resources by Farmers: Some Recent Examples

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The outstanding contributions made by the farmers in the domestication and improvement of Plant Genetic Resources (PGR) in the past are well understood and appreciated. But there is a debate on the role of farmers in PGR development in the present era of predominance of professional plant breeding. We present recent examples of PGR development in rice (Sharbati) and American cotton (Bikaneri Narma and Jhurar) and domestication of a weed *bathu* by the farmers of the Punjab state. The farmers developed these valuable genetic resources in spite of sweeping genetic erosion of PGR in the Punjab state during last about 40 years.

Key Words: *Oryza sativa*, *Gossypium hirsutum*, *Chenopodium album*, Farmers' rights, Benefit sharing

Genetic resources were earlier freely exchanged across political boundaries, and it paid rich dividends. However, with international agreements like Convention on Biological Diversity and World Trade Agreement coming in to force in 1990s, there has been paradigm shift. Genetic resources, earlier considered Heritage of mankind are now sovereign right of the nation and are an intellectual property.

One of the issues that is now a hot topic of discussion and debate is the contribution of farmers in the development, conservation and utilization of Plant Genetic Resources (PGR) and consequent farmers' rights and benefit sharing. Though, there is a general agreement on the stellar role played by the farmers in the domestication and improvement of crop plants, yet, there is no consensus on the farmers' contribution in the present scenario when plant breeding is carried out by professionals and there is a well developed seed industry.

We present recent examples of the development of PGR by the farmers in the Punjab state, the agriculturally most progressive state in the country, where the Green Revolution had started and had the maximum impact. The examples relate to rice (*Oryza sativa* L.) and cotton (*Gossypium hirsutum* L.), the most important food-grain and commercial crops, respectively, in the country. In both crops, Punjab Agricultural University (PAU), Ludhiana, and Indian National Agricultural Research System (NARS) have very strong crop improvement programmes, the competence and contributions of which are well recognized at the national and international levels. In addition, domestication of a weed (*Chenopodium album* L.) is discussed.

With the advent of Green Revolution in 1960s, there was a rapid enhancement of average productivity of wheat (*Triticum aestivum* L.) and rice in the Punjab state (Table 1). The economic returns from the cultivation of these crops increased and that gave a boost to the expansion of the area under their cultivation. Consequently, there has been manifold increase in their production. The Punjab state became grain bowl of the country. Since 1970s it has been the major contributor to the national buffer stock of food-grains comprising wheat and rice, which the National Government maintains to ensure the food-grain supply through the public distribution system. The contribution of the Punjab state has generally been 50 to 75% in wheat and 30 to 45% in rice (Fig. 1), though, the Punjab state has only 1.53% of the geographical area of the country.

Table 1. Area, average productivity and production of rice and wheat in the Punjab state

Crop	Year	Area (m ha)	Productivity (kg/ha)	Production (mt)
Rice	1960-61	0.227	1009	0.229
	2004-05 ^a	2.647	3943	10.437
	2007-08 ^b	2.610	4019	10.489
	Maximum increase (%) ^c	1066	298	4480
Wheat	1960-61	1.400	1244	1.742
	1999-2000 ^d	3.388	4696	15.910
	2007-08 ^e	3.494	4516	15.780
	Maximum increase (%) ^c	149	277	813

^a Rice area was the highest during 2004-05

^b Rice productivity and production were the highest during 2007-08

^c Increase over 1960-61

^d Wheat productivity and production were the highest during 1999-2000

^e Wheat area was the highest during 2007-08

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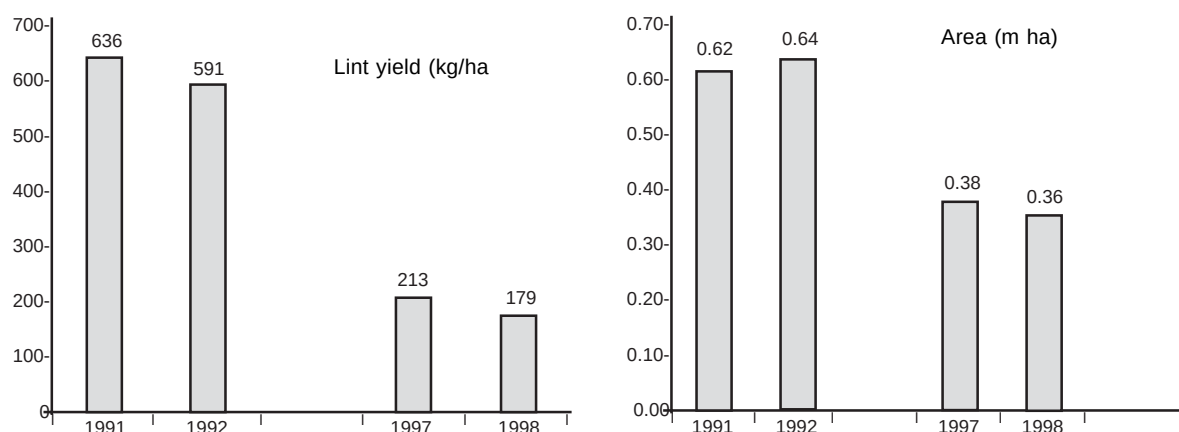


Fig. 1: Lint yield and area under American cotton in Punjab

Total wheat acreage and almost all rice acreage have come under the cultivation of officially released high yielding varieties. In cotton (*Gossypium* spp.), the third most important crop in the state, officially released high yielding varieties occupied more than 90% of the acreage during 2006-07. The spread of high yielding varieties led to sweeping genetic erosion of PGR of these crops, there also being no wild relatives of these crops in the Punjab state and adjoining regions. In spite of that the farmers made significant contributions in the development of PGR in rice and cotton.

Rice

In rice some farmer-bred varieties, such as Sharbati, Govida, Deve Gowda, Gaurav and Tericot were cultivated on a very small area during recent past. Of these, Sharbati has gained importance since 2006. It has long slender grain because of which it got premium price in the market. This accompanied by other desirable traits (early maturity, and tolerance to stresses due to salinity, low moisture and low nitrogen), gave a boost to its cultivation, and the area under it increased without support from any research institution and development agency in the region. During monsoon season of 2007, it was cultivated on about 2% area of 2.6 m ha under rice cultivation in the Punjab state (the cultivation of Sharbati started in mid-1990s). To exploit its desirable traits, Sharbati is being used by the rice breeders of PAU in their hybridization programme.

Cotton

In American cotton (*G. hirsutum*), farmers developed Bikaneri Narma, which was an early maturing germplasm that rapidly spread during 1970s in the cotton belt in North-West India comprising the contiguous area of the states of Punjab, Haryana and Rajasthan. The superiority

of Bikaneri Narma was hesitantly accepted by the plant breeders working in the agricultural universities and research institutes in the region and it was used in various breeding programmes that resulted in the development of improved varieties like F414, H777 and Ganganagar Ageti.

During 1980s, another germplasm Jhurar of American cotton came into prominence. Jhurar was late maturing but had large bolls and high lint yield. In addition, its fibre quality was good due to which it got premium in the market. In spite of an efficient extension education system and excellent rapport of the PAU with the farmers of the Punjab state, the farmers did not respond to the advisories by the scientists to not to cultivate Jhurar. The scientists' apprehension was that the cultivation of Jhurar (because of its long duration) will promote the build up of the population of American bollworm (*Helicoverpa armigera* Huebner), a serious pest of cotton, besides the fact that its cultivation delayed wheat sowing that lowered wheat yield. Unfortunately, the apprehensions came true. There was epidemical incidence of *Helicoverpa* leading to a reduction in yield during late 1990s, the decrease being drastic during the monsoon seasons of 1997 and 1998 (Fig. 2). The lint yields that were as high as about 636 kg/ha during 1991 fell down to about 179 kg/ha during 1998. This drastic reduction in yield led to fall in area under cotton cultivation, the fall being the maximum during 1999 and 2000. During 2000, cotton was cultivated on 0.36 m ha, respectively, as compared to 0.64 m ha during 1992. The cotton cultivation in the North-West India was practically threatened. It picked up thereafter with the intensification of integrated pest management by PAU, and later on by the release of transgenic *Helicoverpa* resistant cultivars. Whatever may be the

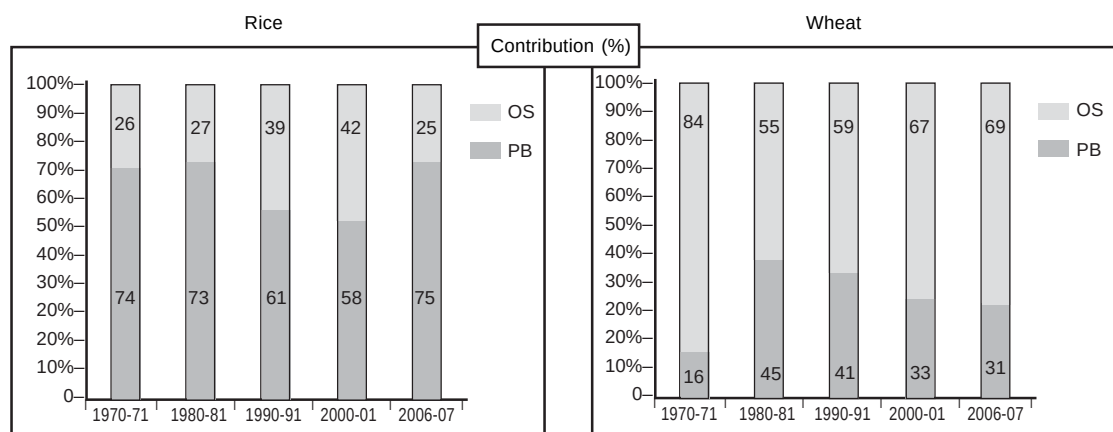


Fig. 2: Contribution of wheat and rice by the Punjab (PB) and other states (OS) to the central pool of food-grains

desirable or undesirable traits of Jhurar, the fact is that Jhurar was an important cultivar during 1985-2000 and the farmer(s) of the Punjab had developed this outstanding germplasm, which possessed some undesirable (late maturity and susceptibility to *Helicoverpa*) traits besides the most desirable ones (high lint yield and good fibre quality).

Bathu

There is a weed, locally known as *bathu* or *bathua* *Chenopodium album* L., in wheat and other winter season crops. *Bathu* is added to the stems and leaves of *Brassica juncea* (L.) Czern. & Coss., *B. rapa* L. and *B. napus* L. while preparing a vegetable, a local delicacy in Punjab known as *saag*. The traditional belief is that *bathu* adds value to taste of *saag*. In fact *bathu* has high percentage of protein (an excellent example of indigenous technical knowledge). With the development of agriculture during and after Green Revolution, all wheat acreage in Punjab came under the treatment of weedicides, and the availability of *bathu* became scarce. On the other hand, the demand went up due to increased population, improved standard of living and popularity of *saag*. To take advantage of the market, some farmers, without any support from any institution, have started cultivation of *bathu* and, thereby, started the process of its domestication.

Source of Germplasm

A valid question arises that from where the farmers obtained the raw material of rice and cotton in the absence of diverse PGR. There can only be guess. In both rice and cotton, farmers do obtain new strains under evaluation in the research programmes of agricultural universities and research institutes in the region, farmers' varieties from the adjoining states, and cotton germplasm from

neighboring country; and some farmer(s) may have worked on the resultant release of genetic variability to develop these farmer's varieties. In cotton, new recombinants do arise as a result of often cross pollination, and there are examples in the official list of cultivars developed by exploiting such variability. But in rice there is a little cross pollination. The most common guess is that the strains developed by some public rice breeding programmes in the region, have been adopted by the farmers. Whatever may be source of germplasm and the protocol of the development, whosoever may be the farmer-breeder(s), and whatsoever may be the merits and demerits of these farmer's varieties, the fact is that outstanding PGR were developed by the farmers and that these farmer's varieties occupied or are occupying appreciable area even in the absence of any support from the agricultural research institutions and development agencies in the region.

Prologue

We have made an attempt to focus on the farmers' role in the development of PGR in the Punjab state, the agriculturally most progressive state, which has no diversity in PGR. It is neither a statement on the contributions of the Indian NARS including PAU nor on the competence of their scientists. It is also not a statement in the context of farmer participatory plant breeding which has its own merits and demerits. The Indian NARS has undoubtedly made outstanding contributions in the development of Indian agriculture and PAU is the cradle of Green Revolution and a front runner among Indian agricultural research institutions in contributing towards the achievement of self-sufficiency in food-grain production and sustenance of national food security.

Morphological and Nutritional Diversity of Some Mungbean Genotypes from Indian Subcontinent

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Seventy eight genotypes of mungbean [*Vigna radiata* (L.) Wilczek] originated from Indian subcontinent were found genetically diverse for morphological characters, level of seed protein and anti-nutritional factors. Genotypes, like, IC103179 and 'Kopergaon' with high protein yield along with low content of trypsin inhibitor were screened. Potential parental combinations for hybridization programme, namely, 'Kopergaon' x TRCM-5-1, 'Kopergaon' x IC103993 were identified through D² analysis for breeding high yielding genotypes with superior quality.

Key Words: Mungbean, Descriptors, D² analysis, Protein content, Trypsin inhibitor content

One of the constraints listed for lack of breakthrough in mungbean production has been non-availability of genetic variability for high yield potential. This was probably due to the utilization of only a few selected genotypes of mungbean in cultivar development programme and underutilization of the genepool of the Indian subcontinent (Gupta *et al.*, 2004). This was supported by the DNA fingerprinting information of Indian cultivars emanated from the investigations of many researchers (Lakhanpaul *et al.*, 2000; Chattopadhyay *et al.*, 2008). Therefore, identification and deployment of diverse genotypes is required for breeding new cultivars in the point of view of food and nutritional security. Total seed protein of mungbean was found to be around 24-25% while soluble protein was reported to be around 20% with huge variability. But protein rich genotypes of pulses may not be always useful to fulfill our nutritional requirements, as it may be associated with some anti-nutritional factors like trypsin inhibitor. The content of trypsin inhibitor in mungbean was found to be low as compared to other pulses (Rosario *et al.*, 1980). But a wide range of variation was found in respect of trypsin inhibitor content.

The present study was aimed at the estimation of genetic diversity on the basis of data of plant seed yield and yield contributing characters among the mungbean lines for two seasons (environments) and to find out the seasonal (environmental) effects on clustering pattern and identify suitable parental combination for improvement programme. Another objective was to identify high yielding and high protein genotypes with minimum level of trypsin inhibitor. Those lines would be regarded as ideal genotypes

which could be either used directly as nutritionally superior varieties or in breeding for quality improvement in mungbean.

Seventy eight accessions of mungbean having diverse origin and nature (Table 1) were grown in the experimental blocks at D-Block Farm, Kalyani of Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India. Those lines belonged to Rajasthan, Gujarat, Delhi, Punjab, Maharashtra, Uttar Pradesh, West Bengal, Tripura and exotic genotypes from Myanmar. The materials were sown in the month of February of the year 2004 and 2005 in Randomized Block Design (RBD) with three replications. The plot size was 5 m², where row to row and plant to plant distances were maintained by 30 cm and 10 cm, respectively.

Two sets of data of seven quantitative characters, collected in two years were subjected to analysis of variance and test of homogeneity was done for each character. Genetic diversity was calculated among germplasm, using data of quantitative characters under two environments, separately, by D² analysis (Mahalanobis, 1936). Clustering of genotypes in the present study was done by Tocher's method (Rao, 1952) where groups were constructed by 'adding' genotypes using D² values. Soluble protein was estimated following Bradford's method (Bradford, 1976) from 50 mg powder of crushed seeds of each sample and colour absorbance reading was taken from Spectrophotometer in 460 nm and protein concentration was measured from standard graph. On the other hand, trypsin inhibitor was estimated from a few selected genotypes, representing all clusters generated by D² analysis from data taken in year 2004.

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100 mg of crushed seed powder of each sample was homogenized with Tris-CaCl₂ buffer having pH 8.1-8.2 for 5 minutes and centrifuged for 30 minutes at 8000 rpm. Aliquot of supernatant was diluted with buffer solution and five different concentration of solution were taken in five tubes. Subsequent analysis of trypsin inhibitor activity (in TIU) was done following the method given by Kakade *et al.* (1974).

Large observable variation was found among genotypes in respect of qualitative characters, like, flowering synchronization, pod and seed colour, etc. IC103788, a landrace from Gujarat was found to be early maturing with synchronous flowering habit. 'Kopergaon', a variety from Maharashtra showed intermediate synchrony and profuse flowering in first flush of flowering. IC39566 with brown colour mature pod, yielded high. On the basis of high GCV, PCV, high heritability and genetic advance over mean and significant positive correlation with seed yield (data not shown), 1000-seed weight and number of pods per plant could be considered as useful selection criteria for identifying pure line or segregating progenies with superior productivity. Among the quantitative characters, the number of branches per plant and plant height were substantially influenced by the changes of environment. 'Kopergaon' was observed to bear highest number of pods while TRCM-5-1 showed largest pod length with larger seed size.

Test of homogeneity revealed that out of seven quantitative characters, five were homogeneous but rest two characters, namely, plant height and number of branches per plant were heterogeneous in nature. Therefore, taking all the characters, pooled D² analysis over the environments could not be done. Under that circumstance, D² analysis on separate data sets have been carried out and tried to identify some distant clusters or genotypes for making potential cross combinations.

Analysis of variances for dispersion and x² test using Wilk's criteria signified the variability among genotypes tested in 2004. The contribution of seed yield per plant was highest (27.87%) followed by 1000-seed weight (25.34%) and number of pods per plant (21.25%) in the estimates of divergence of genotypes. Seven clusters were generated through Tocher's method. Large number of genotypes (46) belonged to cluster-I while other clusters (with suffix II, III, IV, V, VI and VII) contained 16, 8, 2, 1 and 4 genotypes, respectively. Cluster VII possessed genotypes with highest mean seed yield (7.38 g/plant) while cluster V revealed highest mean 1000-seed weight

(46.25 g) with lowest mean plant height (35.7 cm). Cluster V and cluster VI revealed highest inter-cluster distance (17.75) followed by cluster V and VII (16.39). D² value was calculated high between TRCM-5-1 (M-72) belonged to cluster V and S1 (BL x 444D1) (M-63) belonged to cluster VI. Therefore, this combination could be the best in generating useful transgressive segregants. On the other hand 'Kopergaon' (M-66) belonging to cluster VII showed highest D² value against TRCM-5-1 (M-72), belonging to cluster V. That combination also similarly had potentiality to generate enormous beneficial segregates in hybridization.

ANOVA of dispersion revealed the significant level of variability among the same set of genotypes grown in 2005 which was supported by x² test using Wilk's criterion. 1000-seed weight (32.4%) contributed largely to D² values. Again seven clusters were found through Tocher's method. Cluster IV, V, VI and VII contained one each genotype. Cluster II and cluster III contained 14 and 5 genotypes, respectively. Rest 55 genotypes belonged to cluster I. Cluster mean of seed yield per plant (6.37 g) and number of pods/plant (27.1) were highest in cluster V. Cluster III with highest pod length (8.11 cm) and highest 1000 seed weight (42.9 g) revealed the highest inter-cluster distance (12.09) with cluster VII, with lowest 1000-seed weight (6.33 g) and lowest pod length (22.5 cm). Considering the D² values, it was observed that, IC103984 (M-43), in cluster VII and TRCM-5-1 (M-72) in cluster III was most diverse. The second highest inter-cluster distance (11.35) was noted between cluster II and cluster VI. IC103993 (M-45) in cluster VI and 'Kopergaon' (M-66) in cluster II were also found diverse with high D² value. Those two combinations could be efficient for generating variation through hybridization. 'Kopergaon' and Sel1 (Mush x mung) were high yielding and consistently belonged to same group while evaluation for two years. Therefore, crossing between IC103993 and Kopergaon or Sel1 (Mush x mung) would likely to produce diverse segregates.

Significant variability was observed among the genotypes in respect to percent of soluble protein content. The range of the soluble protein content varied from 15% (IC39501) to 25.9% (IC103213) with a mean (20±1.82%) (Table 1). Working with 37 land races of Orissa, Naik *et al.* (2000) found the range of protein content varied from 17.2 to 29.9%. In the present investigation, the per plant protein yield was varied from 0.28 in IC103993 to 1.55 in Kopergaon. The average soluble protein harvest

Table 1. List of mungbean germplasm with their nature, origin, cluster name, estimation of soluble protein content, seed yield, trypsin inhibitor level in selected entries and top ten rankings based on protein yield per plant

Entry No.	Accession number/ name	Nature of germplasm	Origin/place of collection	Cluster by D ² analysis in 2004	Cluster by D ² analysis in 2005	Percentage of protein content	Seed yield per plant (g)	Protein yield per plant (g)	Rank	Trypsin inhibitor (TIU) (selected entries)
M-1	IC39483	Landrace	Kachchh, Gujarat	I	I	17.60	3.015	0.531		—
M-2	IC39484			I	I	21.20	3.96	0.84	1428.57	
M-3	IC39486			I	I	17.20	4.135	0.711	—	
M-4	IC39487			I	I	17.00	2.21	0.376	—	
M-5	IC39488			I	I	21.44	3.38	0.725	—	
M-6	IC39489			I	I	21.48	3.025	0.65	—	
M-7	IC39496			I	I	17.12	3.735	0.639	—	
M-8	IC39499			I	I	24.20	2.365	0.572	—	
M-9	IC39501			I	I	15.00	3.853	0.578	—	
M-10	IC39503			I	I	15.20	2.705	0.411	—	
M-11	IC39504			I	I	20.00	2.975	0.595	—	
M-12	IC39525			I	I	18.24	2.395	0.437	—	
M-13	IC39534			I	III	15.96	3.215	0.513	—	
M-14	IC39535			I	I	21.08	2.978	0.628	—	
M-15	IC39538			I	I	17.08	3.375	0.576	—	
M-16	IC39539			I	I	18.44	3.86	0.712	—	
M-17	IC39540			I	I	21.40	2.563	0.548	—	
M-18	IC39541			III	I	16.72	2.84	0.475	—	
M-19	IC39544			I	I	19.60	3.563	0.698	—	
M-20	IC39547			I	I	15.90	2.945	0.468	—	
M-21	IC39548			I	I	17.72	3.1	0.549	—	
M-22	IC39549			VII	I	24.00	5.56	1.334	II	—
M-23	IC39552			I	I	18.00	2.455	0.442	—	
M-24	IC39561			II	I	19.00	3.575	0.679	—	
M-25	IC39566		Patan, Gujarat	II	V	24.92	4.97	1.239	III	—
M-26	IC39568		I	I	18.52	3.225	0.597	—		
M-27	IC103014		Keda, Gujarat	I	I	18.24	3.82	0.697	—	
M-28	IC103154		Pali, Rajasthan	II	I	17.56	4.365	0.766	—	
M-29	IC103177		Bhilwara, Rajasthan	II	I	23.60	4.538	1.071	VII	—
M-30	IC103179		Bhilwara, Rajasthan	II	I	20.40	4.92	1.004	X	1250.0
M-31	IC103196		Bhilwara, Rajasthan	I	I	22.52	3.37	0.759	—	—
M-32	IC103213		Chitorgarh, Rajasthan	II	I	25.90	4.505	1.167	V	—
M-33	IC103224			I	I	19.28	2.595	0.50	—	—
M-34	IC103238			Banswara, Rajasthan	II	I	20.00	4.635	0.927	—
M-35	IC103300	Kachchh, Gujarat		I	I	22.88	3.165	0.724	—	—
M-36	IC103788		II	I	17.20	3.758	0.646	—	—	
M-37	IC103861		II	I	18.20	2.44	0.444	—	—	
M-38	IC103878		II	I	16.72	2.935	0.491	—	—	
M-39	IC103880	Valbhipur, Gujarat	I	I	18.68	3.07	0.573	—	—	
M-40	IC103973		II	IV	22.36	2.14	0.479	—	—	
M-41	IC103974		III	I	23.24	2.175	0.505	—	—	

Entry No.	Accession number/ name	Nature of germplasm	Origin/place of collection	Cluster by D ² analysis in 2004	Cluster by D ² analysis in 2005	Percentage of protein content	Seed yield per plant (g)	Protein yield per plant (g)	Rank	Trypsin inhibitor (TIU) (selected entries)
M-42	IC103976		Sihor Road, Gujarat	II	I	19.64	3.66	0.719		–
M-43	IC103984		Talaja Road, Gujarat	III	VII	23.92	2.53	0.605		–
M-44	IC103986		Mahuwa Road, Gujarat	III	I	22.72	2.2	0.50		–
M-45	IC103993		Khambhat Road, Gujarat	III	VI	18.64	1.525	0.284		–
M-46	EC16563	Exotic collection		I	I	17.32	2.973	0.515		–
M-47	EC16566			I	II	20.32	2.335	0.474		–
M-48	EC19732		Myanmar	I	III	17.64	4.09	0.721		1500.0
M-49	EC27515			I	I	22.52	3.5	0.788		–
M-50	EC33351			IV	I	23.76	4.065	0.966		1304.34
M-51	Sonamung	Landrace	West Bengal	I	II	20.92	4.335	0.907		–
M-52	Sub-2	Wild (<i>V. radiata</i> var. <i>sublobata</i>)	Rajasthan	III	II	19.64	2.95	0.579		1411.76
M-53	Midnapur local	Landrace	West Bengal	I	II	19.32	3.96	0.765		–
M-54	Malda-95-13			I	I	20.74	3.98	0.825		–
M-55	Radiata-5			I	II	22.48	3.295	0.741		–
M-56	BM-18	Variety	Maharashtra	I	I	19.10	4.27	0.816		–
M-57	PDM-19-257		Uttar Pradesh	I	II	17.80	2.615	0.465		–
M-58	83-m-selection	Selection line	West Bengal	I	II	21.96	2.76	0.606		–
M-59	SML-264	Variety	Punjab	I	II	18.24	3.393	0.619		–
M-60	NP-28		New Delhi	I	II	21.68	4.17	0.904		–
M-61	B-27		West Bengal	I	I	22.32	3.85	0.859		1363.64
M-62	SML-286		Punjab	I	I	24.48	4.22	1.033	IX	–
M-63	S1 (BL x 444DI)	Selection line	West Bengal	VI	II	21.60	5.455	1.178	IV	1463.41
M-64	PS-16	Variety	New Delhi	I	II	17.12	3.778	0.647		–
M-65	Dantan sonamung	Landrace	West Bengal	I	II	18.00	3.84	0.691		–
M-66	Kopergaon	Variety	Maharashtra	VII	II	22.52	6.88	1.549	I	1361.0
M-67	Selli (Mush x mung)	Selection line	West Bengal	VII	II	19.90	5.3	1.055	VIII	1445.78
M-68	Sub-14	Wild (<i>V. radiata</i> var. <i>sublobata</i>)	Rajasthan	III	I	19.40	2.575	0.50		–
M-69	TRCM-1-1	Selection line		II	III	22.00	3.943	0.867		–
M-70	TRCM-3-1			VII	I	19.64	5.895	1.158	VI	–
M-71	TRCM-4-1			II	I	19.16	3.865	0.741		–
M-72	TRCM-5-1			V	III	21.60	3.335	0.720		1333.3
M-73	TRCM-7-1			V	III	21.88	2.94	0.643		–
M-74	TRCM-10-1		Lembucherra, Tripura	II	I	17.24	2.765	0.477		–
M-75	TRCM-16-1			II	I	17.60	4.39	0.773		–
M-76	TRCM-17-1			II	I	20.64	3.69	0.762		–
M-77	TRCM-21-1			I	I	22.48	4.23	0.951		–
M-78	TRCM-22-1-1			I	I	21.24	3.415	0.725		–
Mean						20±1.8	3.54± 0.5			–

per plant was 0.71. The other high protein containing genotypes were IC39566 (24.9), SML-286 (24.48), IC39549 (24), IC39499 (24.2). In respect of high protein yield per plant, the genotype identified were Kopergaon, followed by IC39549 (1.33), IC39566 (1.23), S1 (BL x 444D1) (1.18), IC103213 (1.17), TRCM-3-1 (1.16). On the other hand, the range of trypsin Inhibitor in present study, varied from 1250 to 1500 TIU with an average of 1386 TIU. The content of Trypsin inhibitor level was judged and two genotypes, namely, Kopergaon (1361 TIU) and IC103179 (1250 TIU) were detected to have high protein yield as well as moderate to low level of trypsin inhibitor.

In the research programme, many genotypes were taken from Rajasthan and Gujarat, being one of the probable areas of origin of the species, which were not included earlier in mungbean breeding programme. The germplasm tested in the present study were found highly diverse as revealed from test of significance (X^2 test) and high D^2 values based on phenotypes. Difference in clustering pattern was observed under two environmental situations may be attributed to the change of D^2 values among the genotypes. Variation in clustering pattern in different environments was also observed in mungbean by many workers (Raje and Rao 2001; Patil *et al.*, 2003). The change in the contribution of traits towards the total divergence could be responsible mostly to alter the relationship among the genotypes of mungbean. No relation could be drawn between geographical diversity and morphological diversity. Genotypes with similar origin or place of collection, were found to be placed in different clusters, as observed also in earlier studies in mungbean (Raje and Rao, 2001).

Genotypes were identified for their higher protein yield per plant with low level of antinutritional factor. Those genotypes could be incorporated in the mungbean breeding programme for quality improvement. They were the desirable parents for breeding better quality lines of mungbean. In future, the work on the development of diversity-analysis-model can be taken up where simultaneous incorporation of information on genotypic

and environmental parameters make it able to show the best parental combination for hybridization followed by selection of desired segregants.

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Variability Studies in French Basil (*Ocimum basilicum* L.)

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Variability studies conducted in 30 *Ocimum* genotypes (20 exotic and 10 indigenous) in two environments during *kharif* (rainy season) 2004 and 2005 revealed highly significant variation for all the characters studied. More fresh herbage yield per plant was recorded in the genotypes namely IC333332, EC338785, EC128730 and EC388788. However, high essential oil yield per plant was extracted from genotypes IC110267 and EC388890. The pooled data analysed at phenotypic level revealed that fresh herbage yield per plant had highest positive direct effect with essential oil yield per plant and maximum direct negative effect in essential oil content. The essential oil yield per plant had the highest direct positive effect at phenotypic level on essential oil content followed by fresh herbage yield per plant whereas maximum negative effect was observed in number of flower-whorls per spike and days to seed maturity. The above genotypes performed well in respect of fresh herbage and essential oil yield per plant which could be considered for incorporation in breeding programme to develop an improved variety coupled with high yield potential to enhance the production and productivity.

Key Words: Variability, Path coefficient, Essential oil yield, Fresh herbage yield, *Ocimum basilicum*, Plant traits

Introduction

French basil (*Ocimum basilicum* L.), an annual aromatic herb, belongs to family Lamiaceae having 160 species of *Ocimum* (Balyan and Pushpangadan, 1988). The genus *Ocimum* is widely distributed and extensively cultivated throughout India (Pushpangadan and Bradu, 1995; Verma *et al.*, 1998 and Pandey and Chowdhary, 2002). The diversity in this genus is observed in the tropical rain forests of Africa with the largest 59 reported species followed by the subtropical regions of Africa (19 species), Arabia and Brazil (11 species each), India, Ethiopia and Madagascar with 9, 8 and 7 species, respectively (Pushpangadan and Bradu, 1995). Its aromatic leaves are used fresh and dried as salad dressing, vegetables, flavouring in sauces, vinegar and confectionary products. The essential oil of basil is used to flavour foods, dental and oral products, in fragrances and in traditional rituals and medicines (Guenther, 1949; Simon *et al.*, 1984). Essential oil contains biologically-active constituents that are insecticidal (Deshpande and Tipnis 1977; Chavan and Nikam, 1982; Chogo and Crank 1981), nematocidal (Chatterjee *et al.*, 1982), fungistatic (Reuveni *et al.*, 1984) and antimicrobial properties (Ntezurubanza *et al.*, 1984).

Basil is propagated through seeds. Research work on variability and association of contributing characters for fresh herbage and essential oil yield in this crop is meagre. Present study was undertaken to estimate the variability present in population and direct and indirect effect of

contributing characters on fresh herbage and essential oil yield in indigenous and exotic genotypes of French basil.

Materials and Methods

French basil genotypes (20 exotic, 10 indigenous accessions) were procured from National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India (Table 1). The accessions were grown in a Randomized Block Design (RBD) with three replications at NBPGR Experimental Farm, Issapur, New Delhi, India and Research Farm, Janta Vedic College, Baraut, District Baghpat, Uttar Pradesh, India representing two environments during *Kharif* (rainy season) 2004 and 2005. The nursery was raised during second week of June and 30-day-old seedlings were transplanted. Three rows of each accession having 10 plants per row were transplanted with row to row and plant to plant distance at 45 cm and 30 cm, respectively. Observations were recorded on five randomly selected competitive plants in each genotype for sixteen traits viz. number of primary branches per plant, lamina length (cm), lamina width (cm), leaf-stem ratio, days to flower initiation, number of spikes per plant, spike length (cm), number of flower-whorls per spike, plant height (cm), fresh herbage yield per plant (g), dry herbage yield per plant (g), days to seed maturity, seed yield per plant (g), 1000-seed weight (g), essential oil content (%), essential oil yield per plant (ml) at full-bloom stage. The year wise data was pooled for both the years for both the locations separately, statistically

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Table 1. List of French basil genotypes and their places of collection

S. No.	Genotypes	Country/Area	S.N.	Genotypes	Country/Area
1.	EC174527	Germany	16.	EC388893	USSR
2.	EC128730	Hungary	17.	EC388891	USSR
3.	EC312264	Poland	18.	EC388896	USSR
4.	EC338785	USA	19.	EC388887	USSR
5.	EC387838	USA	20.	EC388889	USSR
6.	EC338788	USA	21.	IC211313	India
7.	EC338794	USA	22.	IC344638	India
8.	EC338782	USA	23.	IC333332	India
9.	EC338772	USA	24.	IC336833	India
10.	EC344638	USA	25.	IC201236	India
11.	EC338773	USA	26.	IC326735	India
12.	EC338775	USA	27.	IC338959	India
13.	EC338776	USA	28.	IC326711	India
14.	EC388890	USSR	29.	IC326732	India
15.	EC388895	USSR	30.	IC110267	India

IC – Indigenous Collection

EC – Exotic Collection

analysed and presented in Table 2 and 3. The differences between genotypes for these characters were tested for significance by using Analysis of Variance (Panse and Sukhatme, 1967). The phenotypic correlation coefficients were partitioned into direct and indirect effects of contributing traits on fresh herbage yield and essential oil yield per plant as suggested by Wright, 1921 and Dewey and Lu (1959) and Pilania *et al.*, (2005).

The herbage was harvested at full-bloom stage and chopped into tiny pieces. The chopped material was hydro-distilled for 3 hours using Clevenger's apparatus for extraction and estimation of essential oil content and yield (Pareek *et al.*, 1982). The essential oil was dried over anhydrous sodium sulphate to remove the moisture.

Results and Discussion

The efficiency of selection largely depends on the magnitude of genetic variability present in the genotype in a plant breeding programme. Genotype EC 338775 was significantly superior over other accessions in respect of number of primary branches per plant followed by EC388788, IC336833, EC387838, IC333332, EC388895, EC338794, EC338772, EC338773 and IC326711 which did not differ significantly among themselves. The accessions EC338785 and EC128730 were statistically at par and significantly superior over other accessions in respect of leaf lamina length. Genotypes EC338775 and EC338776 had significantly more lamina width as compared to other genotypes. The accessions EC 388893 and EC338775 were statistically at par and significantly superior over other accessions in respect of leaf-stem ratio. The genotypes IC326711 was significantly superior

over other genotypes. Genotypes namely, IC336833, EC387838 and EC338794 had significantly more number of spikes per plant and did not differ significantly among themselves. The accessions EC338785 and EC388890 were statistically at par and significantly superior over other accessions in respect of spike length. The least spike length as compared to other accessions was recorded in EC338794. Genotype IC338785 showed significantly more number of flower-whorls per spike as compared to rest of the genotypes. The EC338785 was significantly superior over other accessions in respect of plant height. The genotypes IC333332, EC338785, EC128730 and EC387888 yielded significantly more fresh herbage yield. The genotypes viz. IC333332, EC338785 and EC128730 yielded significantly more and did not differ significantly among themselves. Genotypes EC388887 and IC326711 took significantly more number of days to seed maturity when compared with rest of the genotypes and EC388890, IC338959, EC388788 and EC388893 were significantly at par and took less days to seed maturity as compared to others. The genotype EC388891 and EC 388785 yielded significantly more as compared with other genotypes. The accession IC326711 was significantly superior to other genotypes. The essential oil content was recorded high in IC326732, IC110267, EC388890 and EC388893 which did not differ significantly among themselves. Significantly more essential oil yield were recorded in IC110267 and EC388890 as compared to rest of genotypes.

Fresh Herbage Yield per Plant as Dependent Variable at Phenotypic Level

Results pertaining to direct and indirect effects of fifteen contributing characters on fresh herbage yield per plant at phenotypic level are presented in Table 4. The highest direct positive effect of essential oil yield was recorded with fresh herbage yield followed by dry herbage yield, seed yield, days to seed maturity, lamina width, number of flower-whorls per spike, number of primary branches, plant height and number of spikes. The maximum direct negative effect on fresh herbage yield was observed with essential oil content followed by days to flower initiation, spike length, lamina length, leaf-stem ratio and 1000-seed weight.

Lamina length showed positive correlation with fresh herbage yield and had negative direct effect. The positive correlation was due to indirect positive effect through essential oil yield followed by dry herbage yield and essential oil content. The positive correlation between dry herbage yield and fresh herbage yield was observed and

Table 2. Mean performance of 30 *Ocimum* genotypes for 16 characters in overall pooled data of Issapur and Baraut locations

SN	Genotypes	No. of primary branches per plant	Lamina length (cm)	Lamina width (cm)	Leaf stem ratio	Days to flower initiation	No. of spikes per plant	Spike length (cm)	No. of flower whorls per spike	Plant height (cm)	Fresh herbage yield per plant (g)	Dry herbage yield per plant (g)	Days to seed maturity	Seed yield per plant (g)	1000-seed weight (g)	Essential oil content (%)	Essential oil yield per plant(ml)
1.	IC – 110267	11.33	5.35	2.82	0.86	62.18	55.13	14.41	12.90	46.41	321.76	70.57	157.58	22.01	1.22	0.22	0.71
2.	EC – 338785	10.63	5.89	2.97	1.14	66.82	55.32	23.91	15.28	81.70	344.92	75.71	163.58	43.59	1.64	0.15	0.50
3.	EC – 388890	11.92	5.23	2.68	1.21	60.70	67.67	22.91	14.03	58.65	326.92	70.48	153.58	29.18	1.39	0.22	0.70
4.	EC – 388895	12.18	5.01	2.59	1.13	61.90	69.73	17.08	11.87	57.90	298.92	64.88	155.42	27.24	1.47	0.17	0.50
5.	EC – 388893	11.53	4.83	2.59	1.67	60.85	67.55	15.65	12.65	55.07	238.83	52.84	154.83	34.00	1.32	0.22	0.53
6.	EC – 387838	12.38	5.28	2.90	0.86	61.17	81.27	15.89	12.08	54.28	320.00	70.15	155.00	19.37	1.49	0.14	0.46
7.	EC – 388788	12.45	5.08	2.59	0.84	60.28	77.60	16.09	12.65	56.53	335.00	71.65	153.75	31.67	1.19	0.16	0.53
8.	EC – 338794	12.15	4.73	2.53	1.14	63.02	81.70	10.55	10.85	50.98	330.58	71.42	158.42	28.73	1.47	0.17	0.57
9.	IC – 326735	11.25	5.00	2.54	0.97	62.08	69.53	15.79	12.25	53.45	255.33	55.40	156.83	19.31	1.42	0.18	0.46
10.	IC – 333332	12.23	5.17	2.82	0.83	67.18	75.63	14.49	11.52	50.29	346.25	75.78	163.75	24.64	1.62	0.18	0.62
11.	IC – 336833	12.42	4.99	2.74	0.68	60.57	82.18	13.81	12.07	46.06	287.00	62.54	154.33	12.05	1.27	0.19	0.54
12.	EC – 312264	11.70	4.95	2.72	0.72	63.80	73.87	13.62	12.73	53.28	321.67	70.20	159.58	19.11	1.40	0.17	0.55
13.	EC – 388891	11.62	5.11	2.61	0.99	66.30	69.33	17.66	12.93	58.18	264.92	56.24	163.50	44.65	1.53	0.21	0.56
14.	EC – 388782	11.97	4.88	2.65	0.80	66.47	68.65	14.54	12.50	51.55	313.25	68.42	162.83	29.34	1.47	0.19	0.58
15.	EC-128730	11.48	5.71	2.72	0.82	66.25	69.32	15.51	12.42	56.16	341.25	75.56	158.58	39.45	1.61	0.17	0.59
16.	EC – 338772	12.15	5.00	2.71	1.23	62.80	72.17	14.54	12.53	49.76	297.75	64.63	156.58	14.25	1.25	0.19	0.56
17.	IC – 344638	10.97	5.06	2.69	0.59	63.60	75.02	15.23	12.05	50.38	281.33	60.37	159.17	11.32	1.45	0.18	0.50
18.	EC - 174527	11.47	4.94	2.77	0.86	65.55	79.42	14.40	12.02	50.43	293.17	64.33	156.50	19.67	1.32	0.17	0.49
19.	EC – 387837	11.78	4.47	2.50	1.52	63.92	71.15	12.96	11.83	44.95	275.50	59.96	159.67	10.41	1.38	0.21	0.58
20.	IC – 201233	11.07	4.70	2.74	0.78	62.77	67.57	15.53	11.80	47.71	224.42	49.16	157.58	10.62	1.22	0.17	0.39
21.	EC – 388896	11.30	4.70	2.66	1.07	63.60	67.33	16.69	13.62	51.87	223.58	50.76	157.75	19.68	1.41	0.21	0.47
22.	EC – 338773	12.15	4.44	2.71	0.81	62.63	64.55	20.95	13.12	58.90	304.58	63.95	157.92	19.51	1.52	0.19	0.59
23.	IC – 326711	12.02	5.12	2.87	0.90	72.82	68.58	19.26	13.17	63.14	232.17	50.40	167.08	14.48	1.69	0.21	0.49
24.	EC – 338775	12.98	4.42	3.12	1.67	65.42	79.55	21.49	12.87	57.84	297.75	63.99	156.58	10.82	1.20	0.18	0.54
25.	EC – 338776	11.62	4.55	3.12	1.50	65.22	54.55	22.28	12.30	58.64	258.92	57.58	157.50	11.09	1.62	0.17	0.44
26.	IC – 211313	11.83	4.64	2.61	0.92	66.27	59.00	15.03	12.17	52.38	275.42	61.00	159.75	10.58	1.29	0.16	0.44
27.	IC – 326732	11.77	4.43	2.47	0.62	63.00	67.25	15.87	12.98	46.48	275.75	60.84	157.08	11.16	1.19	0.23	0.63
28.	EC – 388887	11.92	4.91	2.60	0.61	70.38	67.07	11.78	12.05	47.04	296.58	65.68	167.33	10.62	1.31	0.21	0.63
29.	EC – 388889	11.10	4.59	2.59	1.05	64.57	64.62	16.86	12.32	54.83	277.83	60.03	159.75	10.64	1.10	0.16	0.45
30.	IC - 338959	11.38	4.78	2.60	0.85	60.88	69.17	21.43	12.65	59.22	267.92	57.41	153.58	10.78	1.20	0.16	0.44
	Mean	11.76	4.93	2.71	0.99	64.10	69.72	16.54	12.54	54.14	290.98	63.40	158.51	20.67	1.39	0.18	0.53
	Min.	10.63	4.42	2.82	0.59	60.28	54.55	10.55	10.85	44.95	223.58	49.16	153.58	10.41	1.10	0.14	0.39
	Max.	12.98	5.89	2.60	1.67	72.82	82.18	23.91	15.28	81.70	346.25	75.78	167.33	44.65	1.69	0.23	0.71
	SEd.	0.26	0.12	0.06	0.02	0.68	2.12	0.63	0.30	1.36	7.05	1.83	1.22	0.43	0.01	0.01	0.03
	CD at 5%	0.52	0.24	0.12	0.04	1.36	4.24	1.26	0.60	2.72	14.10	3.66	2.44	0.86	0.02	0.02	0.06
	CD at 1%	0.68	0.31	0.16	0.05	1.78	5.55	1.65	0.79	3.56	18.47	4.79	3.20	1.13	0.03	0.03	0.08

Table 3. Analysis of variance (Mean Sum of Squares) for qualitative 16 characters of 30 French basil genotypes

Source of variance	d.f.	No. of primary branches per plant	Lamina length (cm)	Lamina width (cm)	Leaf stem ratio	Days to flower initiation	No. of spikes per plant	Spike length (cm)	No. of flower whorls per spike	Plant height (cm)	Fresh herbage yield per plant (g)	Dry herbage yield per plant (g)	Days to seed maturity	Seed yield per plant (g)	1000-seed weight (g)	Essential oil content (%)	Essential oil yield per plant (ml)
Rep.	2	0.629	0.911	0.038	0.011	0.875	21.063	13.621	0.014	3.688	281	58.625	7.5	0.539	0.0004	0.0004	0.0068
Env.	3	34.724**	1.389**	3.579**	0.014**	28.542	3457.500**	78.148**	3.299**	123.333**	589.607**	341.050**	79.333**	511.990**	0.1785**	0.0013	0.2448**
Genotype	29	3.180**	1.481**	0.319**	1.050**	102.098**	673.128**	133.215**	7.961**	593.996**	151.236**	698.395**	164.724**	1308.368**	0.3014**	0.0067**	0.0731**
(En x G)	87	1.363**	0.269**	0.118**	0.014**	18.958**	129.793**	5.472**	1.672**	14.987*	172.810**	86.412**	6.337	8.101**	0.0031**	0.0013**	0.0153**
Error	232	0.406	0.09	0.019	0.002	2.768	27.059	2.36	0.551	11.093	298.279	20.031	8.865	1.092	0.0011	0.0007	0.0058

* Significant at 5% level; ** Significant at 1% level

Table 4. Direct and indirect effect of genetic variability at phenotypic level on fresh herbage yield in French basil

S. No.	Characters	No. of primary branches per plant	Lamina length (cm)	Lamina width (cm)	Leaf stem ratio	Days to flower initiation	No. of spikes per plant	Spike length (cm)	No. of flower whorls per spike	Plant height (cm)	Dry herbage yield per plant (g)	Days to seed maturity	Seed yield per plant (g)	1000-seed weight (g)	Essential oil content (%)	Essential oil yield per plant (ml)	'r' Fresh herbage yield per plant (g)
1	Number of primary branches per plant	0.029	0.004	-0.001	-0.002	0.001	0.003	0.002	-0.006	-0.003	0.037	-0.007	-0.005	0.000	-0.045	0.168	0.175
2	Lamina length (cm)	-0.005	-0.021	0.008	0.003	-0.006	-0.001	-0.004	0.006	0.009	0.125	0.006	0.033	0.000	0.103	0.137	0.394
3	Lamina width (cm)	-0.001	-0.005	0.037	-0.005	-0.013	0.000	-0.018	0.008	0.010	0.030	0.003	-0.002	0.000	0.179	-0.117	0.105
4	Leaf-stem ratio	0.003	0.004	0.009	-0.020	0.006	-0.001	-0.012	0.003	0.006	-0.046	-0.009	0.007	0.000	-0.041	-0.071	-0.164
5	Days to flower Initiation	-0.001	-0.002	0.010	0.002	-0.050	-0.002	-0.001	0.001	0.005	-0.008	0.043	0.000	-0.001	-0.063	0.019	-0.048
6	Number of spikes per plant	0.009	0.001	0.001	0.002	0.010	0.010	0.013	-0.010	-0.007	0.047	-0.008	-0.003	0.000	0.100	0.007	0.173
7	Spike length (cm)	-0.002	-0.002	0.017	-0.006	-0.002	-0.003	-0.041	0.016	0.020	-0.021	-0.006	0.008	0.000	0.078	-0.116	-0.060
8	Number of flower-whorls per spike	-0.006	-0.004	0.010	-0.002	-0.002	-0.003	-0.021	0.030	0.014	0.018	-0.001	0.017	0.000	-0.078	0.088	0.059
9	Plant height (cm)	-0.003	-0.007	0.013	-0.005	-0.009	-0.003	-0.030	0.015	0.028	0.046	0.006	0.028	-0.001	0.233	-0.150	0.163
11	Dry herbage yield per plant (g)	0.004	-0.009	0.004	0.003	0.001	0.002	0.003	0.002	0.004	0.292	0.001	0.024	0.000	0.276	0.350	0.957
12	Days to seed maturity	-0.004	-0.003	0.002	0.003	-0.043	-0.002	0.005	0.000	0.003	0.005	0.050	0.005	0.000	-0.036	0.015	-0.001
13	Seed yield per plant (g)	-0.002	-0.011	-0.001	-0.002	0.000	0.000	-0.005	0.008	0.013	0.113	0.004	0.062	-0.001	0.037	0.190	0.404
14	1000 seed weight (g)	-0.001	-0.007	0.010	-0.001	-0.022	-0.001	-0.008	0.003	0.012	0.047	0.019	0.028	-0.001	0.049	0.025	0.151
15	Essential oil content (%)	0.001	0.002	-0.007	-0.001	-0.003	-0.001	0.003	0.002	-0.007	-0.087	0.002	-0.003	0.000	-0.924	0.707	-0.314
16	Essential oil yield per plant (ml)	0.005	-0.003	-0.005	0.001	-0.001	0.000	0.005	0.003	-0.004	0.108	0.001	0.013	0.000	-0.689	0.948	0.381

Residual effect (P) = 0.0270

Table 5. Direct and indirect effect of genetic variability at phenotypic level on essential oil yield in French basil

S. No.	Characters	No. of primary branches per plant	Lamina length (cm)	Lamina width (cm)	Leaf stem ratio	Days to flower initiation	No. of spikes per plant	Spike length (cm)	No. of flower whorls per spike	Plant height (cm)	Fresh herbage yield per plant (g)	Dry herbage yield per plant (g)	Days to seed maturity	Seed yield per plant (g)	1000-seed weight (g)	Essential oil content (%)	Essential oil yield per plant (ml)
1	Number of primary branches per plant	-0.001	0.000	0.000	0.000	0.000	-0.005	-0.002	0.007	0.003	0.117	0.006	0.005	0.002	0.000	0.047	0.177
2	Lamina length (cm)	0.000	0.001	-0.003	0.001	0.003	0.001	0.005	-0.007	-0.011	0.263	0.019	-0.004	-0.013	-0.003	-0.107	0.144
3	Lamina width (cm)	0.000	0.000	-0.014	-0.001	0.006	0.000	0.020	-0.009	-0.011	0.070	0.004	-0.002	0.001	-0.002	-0.187	-0.123
4	Leaf-stem ratio	0.000	0.000	-0.003	-0.003	-0.003	0.002	0.013	-0.003	-0.007	-0.109	-0.007	0.006	-0.003	0.000	0.043	-0.075
5	Days to flower Initiation	0.000	0.000	-0.004	0.000	0.024	0.003	0.001	-0.001	-0.006	-0.032	-0.001	-0.028	0.000	-0.003	0.066	0.020
6	Number of spikes per plant	0.000	0.000	0.000	0.000	-0.005	-0.018	-0.014	0.011	0.008	0.115	0.007	0.005	0.001	0.001	-0.104	0.007
7	Spike length (cm)	0.000	0.000	-0.006	-0.001	0.001	0.006	0.044	-0.017	-0.024	-0.040	-0.003	0.004	-0.003	-0.001	-0.081	-0.123
8	Number of flower-whorls per spike	0.000	0.000	-0.004	0.000	0.001	0.006	0.023	-0.033	-0.016	0.040	0.003	0.000	-0.007	-0.001	0.081	0.093
9	Plant height (cm)	0.000	0.000	-0.005	-0.001	0.004	0.004	0.032	-0.017	-0.032	0.109	0.007	-0.004	-0.011	-0.003	-0.243	-0.158
10	Fresh herbage yield per plant (g)	0.000	0.000	-0.001	0.000	-0.001	-0.003	-0.003	-0.002	-0.005	0.668	0.042	0.000	-0.010	-0.001	-0.302	0.381
11	Dry herbage yield per plant (g)	0.000	0.000	-0.001	0.000	-0.001	-0.003	-0.003	-0.002	-0.005	0.639	0.043	-0.001	-0.010	-0.001	-0.288	0.369
12	Days to seed maturity	0.000	0.000	-0.001	0.001	0.021	0.003	-0.005	0.000	-0.004	0.000	0.001	-0.033	-0.002	-0.003	0.037	0.016
13	Seed yield per plant (g)	0.000	0.000	0.001	0.000	0.000	0.001	0.006	-0.009	-0.014	0.270	0.017	-0.002	-0.025	-0.003	-0.039	0.201
14	1000 seed weight (g)	0.000	0.000	-0.004	0.000	0.010	0.002	0.009	-0.004	-0.014	0.101	0.007	-0.012	-0.011	-0.007	-0.051	0.026
15	Essential oil content (%)	0.000	0.000	0.003	0.000	0.002	0.002	-0.004	-0.003	0.008	-0.210	-0.013	-0.001	0.001	0.000	0.961	0.746

Residual effect (P) = 0.019

it also had positive direct effect. This positive correlation was partly due to indirect positive effect of this character via essential oil yield and essential oil content. Seed yield showed positive correlation and direct effect with fresh herbage yield. The positive correlation was due to indirect positive effect of this character via essential oil yield followed by dry herbage yield and essential oil content. Essential oil yield showed positive correlation and direct effect with fresh herbage yield. The positive correlation was partly due to indirect positive effect of this character through dry herbage yield. The residual effect values (0.0270) indicates that the characters studied covered the majority part of variability.

Essential Oil Yield per Plant as Dependent Variable at Phenotypic Level

The path coefficient analysis of essential oil yield per plant as dependent character is presented in Table 5. The results revealed that fresh herbage yield, spike length, dry herbage yield, days to flower initiation and lamina length had the highest direct positive effect on essential oil yield per plant. The maximum direct negative effect was observed for number of flower-whorls per spike, days to seed maturity, plant height, seed yield, number of spikes, lamina width, 1000-seed weight, leaf-stem ratio and number of primary branches.

The correlation and direct effect between fresh herbage yield and essential oil yield was positive. The positive correlation was partly due to indirect positive effect of this character through dry herbage yield. The correlation and direct effect between dry herbage yield and essential oil yield was observed. The positive correlation was partly due to indirect positive effect of this character through fresh herbage yield. The correlation and direct effect between essential oil content and essential oil yield was observed positive. The residual effect values (0.0190) indicates that the characters studied covered the majority part of variability.

Conclusions

It can be concluded from the findings that highly significant variations amongst the various characters existed in different genotypes of French basil under study. The overall pooled analysis of data showed that location had significant effect on all the sixteen characters except essential oil content. The interaction between genotype and environment was also pronounced for all the characters except days to seed maturity. This is apparent that four environments comprised of two cropping seasons at two

locations had greatly influenced the variations in different characters and genotypes. Genotypes namely, IC333332, IC110267, EC388890, EC33885, EC128730 and EC387888 performed well in respect of fresh herbage and essential oil yield per plant when compared with other genotypes. These can be considered in any breeding programme. Like-wise the genotype IC128730 was having multiple desirable features like high fresh herbage yield, high dry matter and high 1000-seed weight. This genotype can also be exploited in *Ocimum* breeding programme.

The pooled analysis of both the locations revealed that essential oil yield had the highest positive effect on fresh herbage yield. The maximum direct negative effect was observed for essential oil content. The essential oil content followed by fresh herbage yield had the highest direct positive effect on essential oil yield and maximum direct negative effect was observed for plant height followed by number of flower-whorls per spike.

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National Herbarium of Cultivated Plants (NHCP): Taxa Added During 2008

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National Herbarium of Cultivated Plants (NHCP) is a major resource for taxonomic and systematic study of plant genetic resources (PGR). For taxa added as herbarium specimens, observations on area and habitat of collection, growth habit, phenology and use of the germplasm are noted on the herbarium sheets. The data checked against reported information aided in recording rarity, endemism, genetic erosion and loss, spread of species etc in 49 taxa added to the NHCP in 2008. Taxa of PGR importance that may be affected by climatic and man-made changes to habitat and morphology can be delineated through these procedures and aid in the use of herbarium resources for measuring species responses to environmental changes.

Key Words: Herbarium, Plant Genetic Resources, Authentication, Diversity, Native, Introduced

Introduction

National Herbarium of Cultivated Plants (NHCP) holds diversity in taxa of economic use/ potential use of the Indian region and is therefore a major resource for identification of PGR. This includes a wide range of taxa belonging to crop species, their wild relatives and weedy types which represent that part of plant diversity which is in a state of flux due to man's changing preferences and priorities. Priority for addition to the NHCP is based not only on floristic records but also on lists of species, both cultivated and wild that are of importance in PGR programmes (Ambasta *et al.*, 1986; Arora and Nayar, 1984; Arora and Nayar, 2007; Arora and Pandey, 1996; Bhatt, 1990; Nayar *et al.*, 2003; Santapau and Henry, 1983). Furthermore, any new introductions and additions to the native and naturalized flora collected from the field are significant for representation in the NHCP. The total holdings of the NHCP amount to 19,799 specimens representative of 3,758 species belonging to 1,430 genera and 262 families.

Holdings of a herbarium, besides its use for identification of species, also aids in collating ethnobotanical and folk importance of species (Cox and Balick, 1994; Jain and Dam, 1979; Schultes, 1960; Von Reis, 1962), working out area of origin/ probable origin (Zeven and de Wet, 1982), as a record of changes in distribution patterns in case of introduced and naturalized species, invasive weeds and a resource for plotting diversity in relation to habitat and climate change (Lane and Jarvis, 2007). For crop species the value of specimens especially of cultivars increases with addition of information on origin, detailed descriptions and images which also

confirms the identity of the plant taxon/ cultivar (RHS, 2009). Since the holdings of NHCP are of potential value as records of PGR diversity, efforts were made to augment data and images on morphological traits, distribution and uses based on collector's data and reported information for taxa added to the NHCP. The standard set of parameters to be considered for the taxa added to the NHCP and the significance of these in interpreting the importance of the herbarium specimen collected and added during the year is brought out in the present paper.

Materials and Methods

Herbarium specimens received and processed for addition to the NHCP were assigned unique identity numbers (HS [Herbarium specimen] numbers). Floristic literature, monographic accounts, checklists and internet resources were used for authentication of taxa represented in the herbarium for its taxonomic status *vis-à-vis* synonymy in plant names; other aspects of morphology, distributional records and uses of species that are relevant for determining their PGR significance were as follows: a) economic value noted during field collection and categorization into crop species, wild relatives of crop plants and minor/ wild economically important or potentially important species; reported uses of plants (Ambasta *et al.*, 1986; Nayar *et al.*, 2003; and Bhatt, 1990) were matched against collector's recorded data; b) habit, period of collection and habitat features noted on the herbarium specimen were checked against reported details in floras (Anon., 2009; Collett, 1921; Press *et al.*, 2000; Wu Zheng-yi & Raven *et al.*, 1994) and features of the plant were checked against descriptions for the taxa; provenance data are mandatory for herbarium specimens as this can aid in

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identification/ confirming the identity of the species using a flora and recording changes if any in flowering and fruiting period. c) areas of collection were checked out against type locality (locality of collection of type specimen), the distributional range of the species, both within the Indian region and outside India, particularly for delineating endemics as well as pinpointing rare or occasional occurrence of the taxa.

The above-mentioned procedure was adopted for taxa represented in the NHCP highlighting the salient features pertaining to the specimen/ taxon and the same was recorded on the specimen sheet for future reference; digital image of specimen/ photographs were included along with the database of information indicated above.

Observations and Results

A total of 49 species were added (97 herbarium specimens) during 2008 which were not represented earlier in the NHCP. Significantly, 44 species were either crop plants (6) or species of minor/ wild economic value including ornamentals, native or introduced (38), the remaining being wild species endemic or native to the area of collection.

Two major sources of specimens were (a) exploration trips, during which collections were made in selected areas, (b) species collected or introduced for their potential importance and raised in experimental plots/ field gene banks, gardens, arboreta, etc. (Table 1). Majority of the specimens added to the NHCP during the year were from

natural habitats (Table 2) in Arunachal Pradesh (6), Delhi (3), Himachal Pradesh (10) and Meghalaya (7) usually collected during the major growing season viz. the monsoon and post-monsoon period (July to November). Equally significant number of species was represented from experimental fields and plots (Table 3) after raising plants from seeds/ propagules or cuttings; this is expected in PGR programmes where the priority for collection is of seeds and propagules/ cuttings and specimens are prepared from material raised in experimental plots.

Crop Plants: Six crop species, not represented earlier in the NHCP (Table 3), were added during the year to the collection of over 350 species already represented in the NHCP:

a) *Species and Cultivars of Crops from Experimental Plots:* Specimens of *Euryale ferox* (makhana), an aquatic species cultivated in sub-tropical eastern parts of Indo-Gangetic plains and extending to East Asia, the Far East and upto the Siberian region, were made from plants raised in Delhi garden; though this area is not a regular area of cultivation, the species flowered and fruited in these conditions similar to the area of commercial cultivation. Furthermore, all parts of the plant were represented in the herbarium specimen (young leaf, mature leaf, flowering node bearing flowers and developing fruits, seeds). Similarly, in *Solanum tuberosum*, flowering specimens were prepared from experimental fields in the hills as 'standard' specimens (RHS, 2009) of different cultivars. Specimen of *Petroselinum crispum* (parsley),

Table 1. Source locality of specimens added to NHCP during 2008

Area of collection: State (District)	Period of collection	Species added (specimens)	Collector
I) Exploration trips for collection of germplasm			
Himachal Pradesh (Kullu, Lahaul & Spiti)	Summer/ Monsoon/ post-monsoon	9 (10)	NBPGR, Shimla
Meghalaya (East Khasi, Shillong)	Summer	4 (4)	NBPGR, Shimla
Arunachal Pradesh (Tawang, West Kameng)	Summer/ Monsoon	6 (6)	NBPGR, Shimla
II) Collection of herbarium specimens			
Delhi and surrounding areas	Summer	3 (3)	NHCP, NBPGR, New Delhi
Himachal Pradesh (Shimla and Solan and adjacent areas)	Summer	8 (11)	NBPGR, Shimla
III Material raised in experimental conditions			
NBPGR Regional Station, Shimla, Himachal Pradesh	Summer and monsoon/ post-monsoon	14 (17)	NBPGR, Shimla
NBPGR Regional Station, Thrissur, Kerala	Post-monsoon	2 (2)	NBPGR, Thrissur
Experimental fields, Central Potato Research Institute, Kufri, Himachal Pradesh	Monsoon	2 (41)	NBPGR, Shimla
Delhi Garden, New Delhi	Summer/ Post-monsoon	1 (3)	NHCP, NBPGR, New Delhi
Summer (March-June) and Monsoon/ post-monsoon (August-October)			

Table 2. Some important wild species added during 2008

S. No.	Species [HS number]	Locality of collection (District, State)	Habitat
1.	<i>Acer sterculiaceum</i> Wall. [19601]	Kullu, HP	Common in deep forest
2.	<i>Aralia cachemirica</i> Decne. [19637]	Kullu, HP	Deep forest; endemic
3.	<i>Corylopsis himalayana</i> Griff. [19503]	East Khasi, Megh.	Frequent along streams
4.	<i>Cotoneaster frigidus</i> Wall. ex Lindl. [19497]	West Kameng, ArP	Riverside; conspicuous when fruiting
5.	<i>Cotula australis</i> Hook.f. [19643]	Shimla, HP	Weed in fields (native of Australia); New record for Shimla
6.	<i>Dichroa febrifuga</i> Lour. [19494]	East Khasi, Megh.	Along roadsides
7.	<i>Ehretia aspera</i> Willd. [19671]	(Ridge) Delhi	Rocky areas; common
8.	<i>Erigeron multiradiatus</i> (DC.) Benth. [19652]	Lahaul & Spiti, HP	Common and gregarious in moist slopes
9.	<i>Helianthus debilis</i> Nutt. var. <i>cucumerifolius</i> (Torr. & A. Gray) Heiser [19575]	Solan, HP	Naturalised upto 1200m; new record for HP
10.	<i>Hypericum choisianum</i> Wall.* [19516]	Kullu, HP	Wild along roadsides
11.	<i>Indigofera dosua</i> Buch.-Ham. [19660]	Shimla, HP	Moist shady places
12.	<i>Juniperus recurva</i> Buch.-Ham. ex D.Don [19646]	Lahaul & Spiti, HP	Forests and thickets, in wetter areas
13.	<i>Jurinea macrocephala</i> (Royle) C.B. Clarke [19658]	Shimla, HP	Rocky crevices
14.	<i>Lindelofia stylosa</i> (Kar. & Kir.) Brand. [19554]	Lahaul & Spiti, HP	Frequent on alpine slopes
15.	<i>Maerua arenaria</i> Hook.f. & Thoms. [19670]	Ridge, Delhi	Rocky areas/ roadside
16.	<i>Olea glandulifera</i> Wall. ex G.Don [19626]	Shimla, HP	Moist ravines
17.	<i>Bistorta affinis</i> (D.Don) Green [19563]	Lahaul & Spiti, HP	Open slopes
18.	<i>Prunus carmesina</i> H. Hara [19514]	East Khasi, Megh.	Deep forest; rare
19.	<i>Prunus phaeosticta</i> (Hance) Maxim. [19486]	East Khasi, Megh.	Along streams; rare
20.	<i>Plectranthus rugosus</i> Wall. ex Benth. [19663]	Shimla, HP	Roadsides
21.	<i>Rubus acuminatus</i> Bl. [19505]	West Kameng, ArP	Dense forest slopes
22.	<i>Rubus alpestris</i> Bl. [19507]	West Kameng, ArP	Deep forest; gigantic plants
23.	<i>Rubus hexagynus</i> Kunth. & Bouche [19501]	West Kameng, ArP	Deep forest; polymorphic
24.	<i>Rubus macilentus</i> Cambess. [19525]	Shimla, HP	Shady moist areas
25.	<i>Salix daphnoides</i> Vill. [19661]	Shimla, HP	Frequent on slopes
26.	<i>Scabiosa speciosa</i> Royle [19544]	Lahaul & Spiti, HP	Along grassy slopes
27.	<i>Tragopogon gracilis</i> D.Don [19592]	Lahaul & Spiti, HP	Rocky dry slopes/ weed in fields
28.	<i>Vaccinium donianum</i> Wight [19491]	Tawang, ArP	Rocky slopes
29.	<i>Zanthoxylum nepalense</i> Babu [19493]	West Kameng, ArP	Open glades in broad-leaved deciduous forests and along tracks; new leaves and flowers appear in spring

ArP.: Arunachal Pradesh; HP: Himachal Pradesh; Megh.: Meghalaya *may also be naturalised

native of Europe and flowering specimens of *Cucurbita maxima* (pumpkin/ winter squash) and *C. argyrosperma* (Cushaw pumpkin) native of South/ Meso-American region were prepared from maintenance plots/ field gene bank collections (Table 3).

b) *Species Collected from Farmers' Fields*: *Allium odorum* (Chinese chives), a predominantly East Asian taxon, cultivated as backyard cultigen for bulbs, leaves and dried seeds was represented from Arunachal Pradesh.

Wild Species Collected from Native Habitats: A total of 28 species were added from exploration trips and collection programmes undertaken in parts of Himachal Pradesh (Lahaul-Spiti, Kullu, Solan and Shimla), parts of north-

eastern region of the country (Meghalaya and Arunachal Pradesh) and from the 'Ridge' area of Delhi (Table 2). Some significant additions identified among the additions were of the following: a) endemic and rare species, *Aralia cachemirica* found in deep forests of Kullu (collected from conservation plot) and comparatively rare species of *Prunus* (*P. carmesina* and *P. phaeosticta*) from the East Khasi Hills of Meghalaya; b) species of native forest areas included *Acer sterculiaceum* from Kullu, Himachal Pradesh and *Rubus* species (*R. acuminatus*, *R. alpestris* and *R. hexagynus*) from West Kameng district of Arunachal Pradesh; high altitude species, *Lindelofia stylosa* and *Erigeron multiradiatus* added from Lahaul and Spiti; c)

Table 3. Plants in field genebank/ raised in experimental conditions/ gardens/ arboreta

S. No.	Source region/ native region	Botanical name [HS number]	Period of collection (Summer/ Monsoon- post monsoon)	Characteristics
TREES AND SHRUBS				
1.	Europe/ Eurasia	¹ <i>Abelia X grandiflora</i> (Andr.) Rehd. (Hybrid of <i>A. chinensis</i> R. Br. and <i>A. uniflora</i> R.Br.) [19534]	Post-monsoon	Ornamental deciduous shrub with white to pink flowers produced in clusters (developed in Italy)
2.		¹ <i>Iris germanica</i> L.* [19656]	Summer	Widespread ornamentals; also naturalised
3.		<i>Rosa tomentosa</i> Sm. [19597]	Summer	Deciduous shrub; irritating hairs around seeds in fleshy hips; also ornamental
4.	North & South America	<i>Magnolia grandiflora</i> L. [19590]	Summer	Medium/ tall tree, large fragrant white flowers/ red seeds in cone-shaped fruits; introduced over wide areas/ conditions
5.		¹ <i>Oenothera biennis</i> L.* [19531]	Summer	Biennial, rosette-forming plant with pale yellow flowers borne on tall spikes; also naturalised
6.		<i>Parkinsonia aculeata</i> L.* [19675]	Summer	Low tree introduced as a source of fibre and fodder; planted along roadsides, with sharp woody spines and yellow showy flowers born in loose racemes and moniliform pods; also naturalised
7.		¹ <i>Passiflora caerulea</i> L. [19564]	Summer	Woody vine with blue to violet floral corona and orange-yellow berry. Popular tropical ornamental
8.		¹ <i>Salvia coccinea</i> Juss.* [19573]	Summer	Widespread ornamentals; also naturalised
9.		¹ <i>Solanum wendlandii</i> Hook.f. [19578]	Summer	Woody vine, fast-growing with bunches of large, deep mauve flowers
10.	South Africa	¹ <i>Crocasmia X crocosmiflora</i> (Lemoine) N.E.Br. [19585]	Summer	Garden hybrid (from <i>C. aurea</i> and <i>C. pottsii</i>); genus native of the grasslands of Cape region
11.	Asia/ East Asia/ China	<i>Maclura tricuspidata</i> Carr. {= <i>Cudrania tricuspidata</i> (Carr.) Bur.} [19522]	Summer	Tree occasionally grown in China for its fruits, similar to mulberry; introduced into various regions
12.	Japan	<i>Viburnum sieboldii</i> Miq. [19654]	Monsoon	Shrub or small tree with stout bristly stem; flowers showy, in clusters and white
13.		<i>Spiraea nipponica</i> Max. [19639]	Summer	Deciduous spreading ornamental shrub, profuse white flowers in corymbs
HERBACEOUS SPECIES				
14.	Asia	<i>Euryale ferox</i> Salisb. [19726]	Post-monsoon	Planted in herbal garden
15.	India, Pakistan	<i>Trigonella emodi</i> Benth. [19634]	Monsoon	Erect herb with yellow flowers and long pods (14mm approx.) raised from seeds
16.	Europe	<i>Petroselinum crispum</i> (Mill.) Airy-Shaw [19523]	Post-monsoon	Herb grown for leaves used as condiment
17.	South America	<i>Solanum tuberosum</i> L. [19734]	Summer	Flowering specimens of cultivars from hills; cultivated in hills/ plains
18.		<i>S. oplocense</i> Hawkes [19765]	Monsoon	Wild species belonging to tuber-bearing <i>S. brevicaulis</i> complex
19.	Mesoamerican region South America	<i>Cucurbita argyrosperma</i> C. Huber [19691]	Summer	Widespread; ovate/ round fruits variable in surface ribbing, stripes & flesh colour (white, yellow or orange); seeds inflated
20.		<i>C. maxima</i> Duch. [19690]	Summer	Highly variable creeping cucurbit

¹Ornamental species *Reported to be naturalized in area of cultivation

introduced and naturalized taxa representing new additions to the local flora, namely *Helianthus debilis* subsp. *cucumerifolius* (Solan district, Himachal Pradesh) and *Cotula australis* (Shimla, Himachal Pradesh).

Checking the area of collection of the wild species against the distributional range (Zeven and de Wet, 1982; GRIN, 2009) showed that species collected were to a large extent native to India. Species from the north eastern region were either localized to the area of collection or extended to the Sino-Japanese or South East Asian region; species collected from the north western region were mostly widely distributed within the Himalayan region or extending upto Iran and Afghanistan and in some cases upto North Africa.

Ornamentals and other Exotic Species: A total of 15 species of woody and herbaceous ornamentals and others of potential economic value (Table 3): a) *Maclura tricuspidata*, native of China (occasionally cultivated), closely similar to mulberry and introduced into arboreta around the world was maintained at Shimla, Himachal Pradesh; b) woody trees, shrubs and climbers representative of summer flowering ornamentals of Shimla district, Himachal Pradesh included *Spiraea nipponica* and *Viburnum sieboldii* native to Japan, *Abelia X grandiflora* of European region, *Solanum wendlandii* native of Costa Rica and *Crocosmia X crocosmiflora* (ornamental developed in the European region from South African species); c) *Magnolia grandiflora*, *Passiflora caerulea* and *Parkinsonia aculeata*, introduced from the North/South American region and summer flowering, were widespread trees of the sub-tropical regions of India; d) species of the European region, *Scabiosa speciosa* and polymorphic *Bistorta affinis* flowered during the monsoon/post-monsoon period in the comparatively cooler climates in Lahaul and Spiti, Himachal Pradesh. Herbaceous species of ornamentals which were added during the year (5) invariably were noted to have the tendency to be naturalized.

Conclusions

Analysing the importance of material added to the herbarium through a synoptic interpretation of field and reported information was found to aid in highlighting significant additions. These were mostly of economically useful taxa (nearly ninety percent); the remaining were native locally available species of natural habitats. Process of authentication and checking out additional information such as detailed descriptions, references to images and

distributional notes, origin or parentage and any other information not only helped to check the identity of the plant specimens but was also valuable for working out endemic and rare types especially in the north western and north eastern regions of high endemism and native diversity. Taxa which need to be taken up for further study could be identified viz. perennials such as the wild relatives of *Prunus*, *Rubus* and *Rosa*, polymorphic taxa such as *Plectranthus rugosus* and taxonomically confusing taxa such as *Bistorta affinis*; all parts of the plant need to be represented in these species for further taxonomic and systematic studies.

Another important component was of introduced species, both cultivated and wild; *Solanum oplocense* represented one of the close relatives of potato introduced for breeding programmes of potato. Over 500 introduced species of wild relatives of crop plants and potentially important taxa by the NBPGR (Nayar *et al.*, 2008) constitute a priority group for representation in the NHCP. Plant habit, period of flowering/ fruiting and area of origin showed links to potential for getting naturalized particularly in ornamental introductions, an aspect requiring detailed survey based on a wider range of taxa.

Finally, the analysis helped to highlight the focal aspects for additions to the NHCP, viz. details of habitat of collection (natural or man-made) for wild species and cultigens and representation of all parts/ stages of growth of the plant (flowering, fruiting and seeds) which aid in correct identification of the material and taxonomic study of crops/ potential domesticates and related wild species.

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