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CONTENTS

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Adaptation Pattern and Genetic Potential of Indian Pearl Millet Named Landraces Conserved at the ICRISAT Genebank

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A total of 692 accessions belonging to 32 named landraces, adapted to a latitude range of 8.8°N to 30.90°N and originating in nine provinces of India were used to study their adaptation pattern and genetic potential. A clear pattern regarding association of latitude, climate at collection sites, adaptation of landraces and the agronomic performance was observed. Hierarchical cluster analysis using eight agronomic traits resulted in four clusters of accessions. Cluster 1 accessions flowered early and cluster 3 flowered late. Cluster 1 and cluster 4 accessions produced more tillers. Important sources identified were IP 13465 for early flowering and short height; IP 15273 for high tillering, high seed iron and zinc content and downy mildew resistance; IP 3531 for high seed protein content, downy mildew and rust resistance; IP 15285, IP 15288, IP 15290, IP 15301, and IP 15351 for high tillering.

Key Words: Adaptation pattern, Diversity, Genebank conservation, ICRISAT landraces, Pearl millet

In India, pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the fifth most important cereal after rice, wheat, maize and sorghum and it is grown mainly for food, feed and fodder. Endowed with richness of landraces, India is the largest producer of pearl millet, both in terms of area (about 8.78 million hectares) and production (10.28 million tons) (Ministry of Agriculture, 2014). Major pearl millet growing states in India include Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana.

Landrace is a dynamic population of a cultivated plant that has a historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming system (Tania *et al*., 2005). Generally, traditional farmers or ethnic groups recognize and distinguish and name the landraces according to their maturity (early, medium and late) and grain characteristics (panicle shape and length, presence of bristles, grain size and color) or some other unique trait found in the material. Although yield is not high, the stability of named landraces in the face of adverse climatic conditions is typically higher than improved cultivars. Such named landraces developed through natural selection coupled with human selection over decades, offer a valuable genepool for future crop improvement programmes. There are many specific examples of the contribution of pearl millet named landraces or folk varieties towards the development of modern varieties. Best example is ICTP 8203, a variety developed at ICRISAT using *Iniadi* landrace originating in Togo. It was promising for early maturity, high seed yield, large seed size and downy mildew resistance and, has been released in India and Africa (Rai *et al*., 2008). Likewise, an S_2 progeny of ICTP 8203 was identified as maintainer of A_1 cytoplasmic-nuclear male sterility system in pearl millet (Rai *et al*., 2008). Further inbreeding and selection led to the development of maintainer line 863B and its male-sterile counterpart 863A, which is a seed parent for three commercial hybrids in India (Rai *et al*., 2008). These examples indicate that the landrace *Iniadi* has unique combination of several useful traits, including high levels of resistance to multiple pathotypes of downy mildew, tolerance to terminal drought, high quality stover yield,

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higher levels of grain iron and zinc (Rai *et al*., 2008). Safeguarding and exploitation of these valuable resources along with related information is essential to enhance their utilization for sustainable pearl millet improvement. The genebank at ICRISAT conserves the world's largest collection of 22,888 pearl millet germplasm accessions from 51 countries including 6,610 accessions from India. Present study aimed at assessing the adaptation pattern and genetic potential of 32 most common pearl millet named landraces originated in India and assembled at ICRISAT genebank, to exploit their potential for pearl millet improvement, globally.

Materials and Methods

Passport and characterization data of the pearl millet named landraces originated in India and conserved at the ICRISAT genebank, were used in the present study. Named landraces, having georeferenced data and at least five or more accessions, were considered for the present study. A final set of 692 accessions belonging to 32 named landraces (hereafter called landraces) were used in the present study.

Landrace accessions were grown in batches of 500 to 1000 every year at ICRISAT, Patancheru, India in alfisols during the rainy and postrainy seasons from 1974 through 2013, as and when the new germplasm was added to the collection. During rainy season, accessions were sown in June and harvested in October/November. However, during postrainy season, accessions were sown in November and harvested in March of subsequent year. Two different seasonal conditions are typical to the semi-arid regions. The day length decreases from 13.20 h (in June) to 11.70 h (in October) with a mean of 12.6 h during rainy season and increases from 11.10 h (in December) to 12.00 h (in March) during postrainy season. The monthly mean minimum temperature varies from 22.4ºC (in June) to 20.4ºC (in October) and the monthly mean maximum temperature varied from 32.8ºC (in June) to 28.2ºC (in August) during rainy season. During postrainy season, monthly mean minimum temperature increased from 11.4ºC (in December) to 19.4ºC (in March) and the mean maximum temperature increased from 27.8ºC (in December) to 33.1ºC (in March). The mean annual rainfall at Patancheru was 877 mm. Plot size consisted two rows of 4 m length, spaced at 75 cm between rows and 10 cm between plants within a row to accommodate 80 plants per plot. Accessions were randomized in all the evaluations with a control at every 20 test entries. Fertilizers were applied at the rate of 100 kg N and 40 kg P_2O_5 ha^{-1}. Need based irrigations were given during rainy season, while the crop was irrigated at regular intervals during postrainy season. The crop was protected from weeds, pests and diseases.

Data were recorded on eight quantitative (days to 50% flowering, plant height, panicle length and thickness during rainy and postrainy seasons, total and productive tillers per plant, panicle exsertion and 1000 seed weight) and eight qualitative (panicle shape, panicle density, bristle length, green fodder yield potential, seed yield potential, seed shape and color, and endosperm texture) traits (IBPGR and ICRISAT, 1993). Days to 50% flowering, plant height, panicle length and thickness were recorded both during rainy and postrainy seasons. All other traits, except seed traits were recorded during rainy season. Seed traits were recorded during postrainy season. Emergence of stigma in 50% plants in a plot was recorded as days to 50% flowering. Mean height of five representative plants from base to the tip of plant was recorded in centimeters as plant height. Mean number of total and productive tillers was recorded in three plants. Distance between the ligule of the flag leaf and the base of panicle was recorded in centimeters as panicle exsertion. Panicle density was scored on 1-9 scale. Score 1-3 was considered as loose panicle, score 4-6 as intermediate and score 7-9 as compact panicle. Length of bristles was scored on 1-9 scale. Score 1-3 as short (bristles below the level of the apex of the seed), score 4-6 as medium (bristle length between 0 and 2 cm above the seeds) and score 7-9 as long (bristles longer than 2 cm above the seed). Endosperm texture was scored on 1-9 scale; score 1-3 as mostly corneous, 4-6 as partly corneous and 7-9 as mostly starchy. Green fodder yield potential was scored on 1-9 scale (1 being the poorest yielder and 9 as the excellent yielder) considering plant height, tillering, leafiness, etc. Seed yield potential was scored on 1-9 scale (1 being the poorest and 9 as the excellent yielder) considering number of productive tillers per plant, panicle size and seed size.

The high resolution (1 km) climatic data such as monthly mean (over past 30 years) day length, minimum and maximum temperature and mean annual rainfall

for each collection site, was retrieved from http://www.worldclim.org/current using the spatial analyst extension in ArcGIS® software (Hijmans *et al*., 2005). Averaged over crop season (June to November during rainy and November to March during postrainy season) at collection sites of landraces, the minimum, maximum and mean temperature, day length and mean annual rainfall for each landrace was estimated.

In the present study, photoperiod and temperature responses were defined by the difference in flowering during rainy and postrainy season (Upadhyaya *et al*. 2012). When the measurements (days to 50% flowering) are high during the relatively cool short-day postrainy season, the accession was considered as temperature sensitive and requires higher temperature for flowering. When the measurements are high during the warm long-day rainy season, then the accession was considered as photoperiod sensitive and requires short days for flowering. When there was no difference in measurements (rainy-postrainy= 0 days), then the accessions were considered as insensitive to both temperature and photoperiod. Database for disease reaction of pearl millet germplasm at ICRISAT genebank indicated that only a limited number of landrace accessions from India were evaluated for downy mildew, rust and nutritional traits such as seed protein, iron and zinc content, at ICRISAT, Patancheru, India (IBPGR and ICRISAT, 1993). Using the available data on nutritional traits and disease severity percentage, promising accessions within each landrace were identified for utilization in pearl millet improvement programmes.

Agronomic data of individual accessions were standardized by subtracting the mean value of the trait from each observation and subsequently dividing by its standard deviation. This resulted in standardized values for each trait with an average value of 0 and standardized deviation of 1 or less. The standardized values were used to perform principal component analysis (PCA) on Genstat 14 release (VSN International, 2010). Cluster analysis (Ward, 1963) was performed using scores of first five principal components (PCs) to group different landraces. Each cluster of landraces were plotted individually on political map of India. Mean, range and variances were calculated for eight quantitative characters in each cluster. The cluster means of different traits were compared using the Newman-Keuls procedure (Newman, 1939; Keuls, 1952). Homogeneity of phenotypic variances was tested by Levene's test (Levene, 1960). Shannon and Weaver (1949) diversity index (H`) was used to measure and compare the phenotypic diversity for all eight traits in each cluster. Phenotypic correlations were estimated among all quantitative characters and their significance was tested (Snedecor and Cochran, 1980). Frequencies were estimated for each class of qualitative traits.

Results

The Collection

The analysis of passport information of world collection of pearl millet germplasm at ICRISAT genebank, India, indicated India as the major source of germplasm with 6,610 accessions. The collection from India includes 6,064 landraces, 403 breeding materials, one advanced cultivar and 142 accessions of wild relatives. A total of 692 accessions belonging to 32 landraces under study had originated in nine provinces of India (Table 1).

Cluster Analysis

Principal Component Analysis (PCA) carried out using standardized data of eight quantitative traits captured 92.3% of total variation from the first five principal components (PCs). A hierarchical cluster analysis conducted on the scores of the first five PCs resulted in four clusters of landraces (Fig. 1 and Table 1). Cluster 1 consisted of 217 accessions of six landraces (Bchwaddawana, Chadi local, Desert type, Gullisita, Mahudo and Pitta ganti). Cluster 2 consisted of 150 accessions of four landraces (Barmer local, Jakhrana, Karauli and Sulkania), all from Rajasthan. Cluster 3 consisted of 121 accessions of seven landraces, all from Tamil Nadu. Cluster 4 consisted of 204 accessions of 15 landraces originating in Tamil Nadu (8), Andhra Pradesh (2), Gujarat (2), Maharashtra (4) and Uttar Pradesh (1).

Adaptation of Landraces

The collection under study was adapted to a latitude range of 8.8^{o} N in Tamil Nadu to 30.90^{o} N in Punjab (Fig. 1). Landraces of cluster 1 were adapted to a latitude range of 14.53^{o} N to 30.90^{o} N with mean latitude of 24.64^{o} N; those of cluster 2 from 24.00^{o} N to 29.55^{o} N with mean latitude of 27.34^{o} N; those of cluster 3 from 8.79^{o} N to 11.90^{o} N with a mean of 9.97^{o} N and

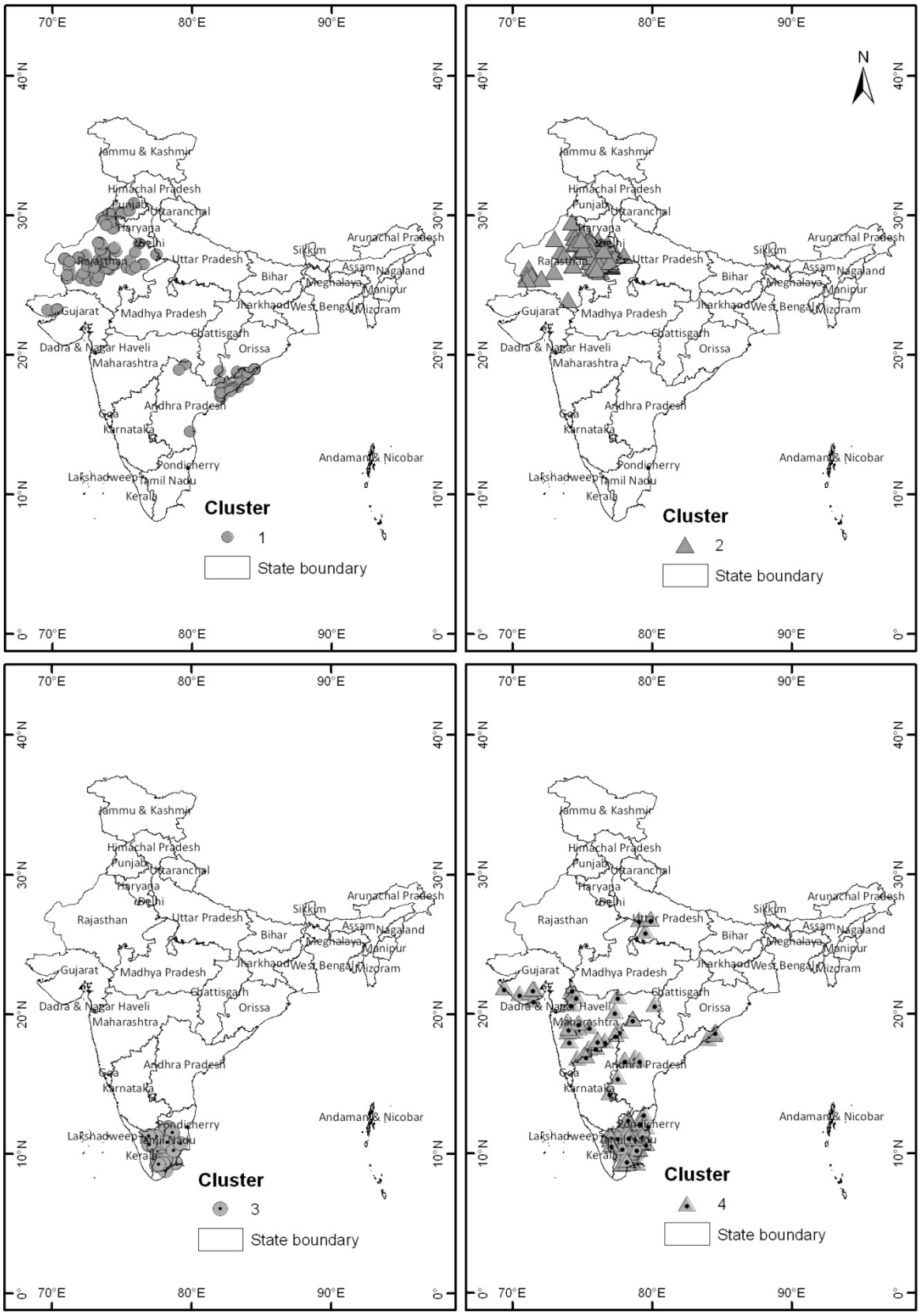

Fig. 1. Geographical adaptation of pearl millet named landraces from India, conserved at ICRISAT, India

Table 1. Pearl millet named landraces from different provinces of India, conserved at ICRISAT, India

Landrace	Cl no.	Andhra Pradesh	Gujarat	Haryana	Maharashtra	Orissa	Punjab	Rajasthan	Tamil Nadu	Uttar Pradesh	Total
Bchwaddawana	1						10				10
Chadi local	1			2			1	82			85
Desert type	1							22			22
Gullisita	1						7	21			28
Mahudo	1		6								6
Pitta ganti	1	64				2					66
Barmer local	2							41			41
Jakhrana	2			6				5			11
Karauli	2							89		2	91
Sulkhania	2							7			7
Eravai	3								8		8
Kala cumbu	3								6		6
Manavari	3								17		17
Mathuravellai	3								39		39
Peria cumbu	3								24		24
Podi cumbu	3								22		22
Umi	3								5		5
Adchi	4				7						7
Amreli	4		5								5
Arisi	4								16		16
Babapuri	4		8		1						9
Gaorani	4				27						27
Kattu cumbu	4								44		44
Kullan	4								20		20
Mainpuri	4									5	5
Nattu cumbu	4								17		17
Oomi cumbu	4								8		8
Pedda sajja	4	11									11
Pokku cumbu	4								9		9
Pottu cumbu	4								7		7
Sadguru bajra	4	5			4						9
Vellai	4								10		10
No. of accessions		80	19	8	39	2	18	267	252	7	692

those of cluster 4 from 9.28^{o} N to 26.83^{o} N with a mean latitude of 13.93^{o} N. Mean latitudes of different clusters indicated that the landraces of cluster 1 and cluster 2 are from higher latitudes when compared to those of cluster 3 and cluster 4. Out of 32 named landraces, a maximum of 15 landraces were adapted in Tamil Nadu followed by seven in Rajasthan, four in Maharashtra, three each in Andhra Pradesh, Gujarat and Punjab, two each in Haryana and Uttar Pradesh and one in Orissa (Table 1). Representation of individual landraces with more than 20 accessions in the collection suggested wide adaptation and extensive collection of Pitta ganti in Andhra Pradesh; Gaorani in Maharashtra; Barmer local, Chadi local, Desert type, Gullisita and Karauli in Rajasthan; Kattu cumbu, Mathuravellai, Peria cumbu and Podi cumbu in Tamil Nadu.

Diversity in the Collection

Qualitative traits

***Panicle shape*:** Out of nine panicle shapes in the world collection, six (cylindrical, conical, spindle, candle, lanceolate and oblanceolate) were found in the collection under study (IBPGR and ICRISAT, 1993). Cluster 1 and cluster 2 were the predominant sources for cylindrical shape, whereas cluster 3 and cluster 4 for candle shape with a frequency of more than 50% (data not given).

***Panicle density*:** A maximum of 36% accessions scored 5 and only three accessions scored 7. IP 3144 and IP 3366 of Karauli scored 8 and were promising for compact panicle pearl millet.

***Bristle length*:** 94% accessions produced short bristles (bristles below the level of the apex of the seed) with a score of 1-3. IP 3278 of Gullisita and IP 3527 of Mathuravellai scoring 7 were the promising sources for bird-tolerant pearl millet.

***Seed shape*:** Five seed shapes (obovate, oblanceolate, elliptical, hexagonal and globular) were found in the collection. Oblanceolate shape was predominant in cluster 2, 3 and 4 and elliptical shape was predominant in cluster 1.

***Seed color*:** Three (grey, deep grey and grey brown) of the 10 colour classes were found. Accessions producing grey seeds were predominant in cluster 2, cluster 3 and cluster 4, whereas those producing grey-brown seeds were predominant in cluster 1.

***Endosperm texture*:** 26% of total accessions had corneous endosperm, a rich source of protein.

***Green fodder yield potential*:** Sixty-six per cent accessions had the score of more than 5 for fodder yield, indicating the intense selection by farmers for high fodder yielding pearl millet. Among the clusters, 71% accessions of cluster 1, 13% accessions of cluster 2, 49% each of cluster 3 and cluster 4 had a score of 7. Nineteen accessions belonging to 11 landraces had score of 8. IP 3188 and 3246 of Chadi local; IP 3173, IP 3190, IP 3245 of Desert type; IP 3517 of Eravai; IP 3586 of Kala cumbu; IP 3582 of Mathuravellai; IP 1305 and IP 3610 of Peria cumbu; IP 9101 and IP 17877 of Adchi; IP 1174 of Amreli; IP 9074 and IP 9076 of Gaorani; IP 3479, IP 3596 and IP 16238 of Kattu cumbu and IP 16283 of Sadguru bajra had a score of 8 and these were promising for high green fodder yielding pearl millet.

***Seed yield potential*:** Only nine (1%) accessions scored 8 for seed yield. IP 3231, IP 3233, IP 3234 and IP 3239 of Chadi local; IP 3244 of Desert type; IP 9076 and IP 9119 of Gaorani; IP 3479 of Kattu cumbu and IP 3477 of Peria cumbu were identified as promising for high seed yield.

Quantitative traits

***Range*:** Range of variation among the clusters for different agronomic traits indicated cluster 1 for early flowering (<50 days), short plant height and panicle length during postrainy season; cluster 2 for panicle length during rainy season; cluster 3 for early flowering (<50 days) during rainy season and cluster 4 for short plant height during postrainy season, total and productive tillers, high panicle exsertion during rainy season, high panicle thickness during both seasons and high 1000 seed weight during postrainy season, showed wide variation (Table 2).

Range of variation for individual landraces indicated that the landraces from lower latitudes (9^{o}-13^{o}N) varied widely for days to flowering during rainy and postrainy season and plant height during rainy season when compared to those from higher latitudes (Table 3). Pitta ganti, Babapuri and Adchi for flowering during rainy season; Bchwaddawana and Pitta ganti for short height (<135 cm) and Mathuravellai and Eravai (>390 cm) for tall height during rainy season; Oomi cumbu, Pokku cumbu, Vellai, Arisi, Mathuravellai, Nattu cumbu and Podi cumbu for short height (<100 cm) and Kattu cumbu (335 cm) and Karauli (270 cm) for tall height during postrainy season; Kattu cumbu and Chadi local, Pitta ganti for total tillers per plant; Kattu cumbu, Manavari and Pokku cumbu for productive tillers; Jakhrana, Karauli, Gullisita and Sulkania for long panicles (40 cm) during rainy and postrainy seasons; Gaorani, Jakhrana and Karauli for relatively larger seeds (>10 g/ 1000 seeds) were the important sources (Table 3).

***Means*:** Mean values tested by Newman-Keuls test indicated significant differences among the clusters for one or more traits under study (Table 2). Accessions of cluster 1 flowered early during both the seasons, grew

Table 2. Range, mean* and variance for various traits of Indian pearl millet landraces in different clusters, evaluated at ICRISAT, India**

Trait		Cluster 1	Cluster 2	Cluster 3	Cluster 4	F value	P
Days to 50% flowering-R	Range	38-74	46-69	47-135	43-119		
	Mean	57.0d	61.0c	93.1a	68.0b		
	Variance	46.9	19.9	257.8	318.0	44.2	<0.0001**
Days to 50% flowering-PR	Range	50-101	52-78	55-94	45-95		
	Mean	67.4c	70.7b	73.8a	67.9c		
	Variance	37.6	26.1	48.6	64.5	5.7	<0.0001**
Plant height (cm)-R	Range	120-269	155-299	200-416	146-384		
	Mean	194.4d	231.7c	328.9a	245.2b		
	Variance	871.0	867.0	1820.0	3656.0	45.8	<0.0001**
Plant height (cm)-PR	Range	100-255	110-270	88-210	60-335		
	Mean	184.5b	205.9a	143.4c	149.3c		
	Variance	740.3	688.6	446.9	965.1	2.4	0.071 NS
Total tillers (no.)	Range	1-18.5	1-7.7	1-16.5	1-18.5		
	Mean	4.4a	2.9b	3.3b	4.6a		
	Variance	5.5	1.2	4.8	13.0	9.0	<0.0001**
Productive tillers (no.)	Range	1-8	1-6.8	1-10.7	1-12.5		
	Mean	3.1a	2.6b	2.0c	3.3a		
	Variance	1.9	0.8	2.0	5.8	12.0	<0.0001**
Panicle exsertion (cm)	Range	-8-15	-3-15	-10-14	-9-17		
	Mean	7.1b	6.3b	4.7c	8.0a		
	Variance	13.0	11.0	14.4	18.0	1.7	0.1583 NS
Panicle length (cm)-R	Range	14-43	19-50	15-40	12-35		
	Mean	21.4c	30.3a	25.4b	21.5c		
	Variance	12.4	35.0	17.6	22.0	11.6	<0.0001**
Panicle length (cm)-PR	Range	11-50	13-45	11-27	8-31		
	Mean	16.8b	22.9a	16.9b	17.5b		
	Variance	15.5	23.7	12.0	17.3	1.4	0.256 NS
Panicle thickness (mm)-R	Range	13-30	15-28	14-27	12-35		
	Mean	19.6b	19.4b	18.9b	20.8a		
	Variance	9.2	5.0	6.8	17.5	20.8	<0.0001**
Panicle thickness (mm)-PR	Range	12-30	12-28	11-25	10-31		
	Mean	18.4b	19.7a	15.2c	19.1ba		
	Variance	10.9	8.2	8.3	24.2	28.6	<0.0001**
1000-Seed weight (g)	Range	3.2-9.7	4.1-10.55	2.5-8.5	2-10.7		
	Mean	6.8b	7.7a	5.5c	6.3b		
	Variance	1.6	1.1	1.2	3.8	35.4	<0.0001**

* Means were tested by using student Newman-Keuls test; means followed by different letter are significantly different at p=0.05,

** variances were tested using Leven's test

Table 3. Range of variation for different traits of pearl millet named landraces from India, evaluated at ICRISAT, India

Landrace	Cl. no.	Latitude range	Days to 50% flowering-R	Days to 50% flowering-PR	Plant height (cm)-R	Plant height (cm)-PR	Total tillers (no)	Productive tillers (no)	Panicle exsertion (cm)	Panicle length (cm)-R	Panicle length (cm)-PR	Panicle thickness (mm)-R	Panicle thickness (mm)-PR	1000-Seed weight (g)
Bchwaddawana	1	30.2	45-55	59-84	120-210	145-185	1.3-2.6	1.0-2.6	-5-8	21-28	17-27	17-25	15-28	3.35-8
Chadi local	1	25.3-30.9	46-67	50-77	150-240	155-215	2.0-18.5	1.4-8.0	2-12	16-29	12-25	13-30	12-25	5.0-9.0
Desert type	1	26.3-29.9	49-68	51-76	140-216	153-210	1.8-9.2	1.8-6.6	2-14	16-23	11-21	14-20	13-18	3.2-8.1
Gullisita	1	26.5-30.9	53-70	58-76	180-269	130-255	1.4-5.4	1.2-4.2	-8-15	20-43	13-50	15-28	13-30	4.9-8.6
Mahudo	1	23.1-23.3	51-74	60-72	137-233	175-230	1.0-4.8	1.0-3.6	0-8	15-31	15-27	19-25	18-30	6.4-9.7
Pitta ganti	1	14.5-19.3	38-72	58-101	130-260	100-240	1.6-18.0	1.0-7.2	-5-14	14-27	11-21	15-26	13-24	3.7-9.2
Barmer local	2	25.5-28.8	50-69	59-78	155-290	155-245	2.0-7.4	1.2-6.8	5-14	20-39	13-28	15-23	13-24	4.1-9.1
Jakhrana	2	26.3-28.3	46-58	61-76	165-232	110-210	1.0-4.0	1.0-4.0	-2-15	19-50	18-45	16-28	17-24	6.1-10.6
Karauli	2	24.0-29.6	53-69	52-77	187-299	140-270	1.2-7.7	1.2-4.8	-3-13	22-45	15-32	15-25	12-28	5.2-10.1
Sulkhania	2	27.6-28.3	53-65	59-76	222-280	160-225	2.0-3.2	1.8-3.0	5-10	26-42	17-31	15-20	15-22	7.0-8.6
Eravai	3	9.2-10.9	80-108	62-91	225-394	102-154	1.6-4.0	1.0-3.0	0-5	25-30	14-25	16-21	12-20	3.8-8.1
Kala cumbu	3	9.3-10.4	47-135	63-92	210-342	122-170	2.2-5.5	1.2-4.4	-10-12	21-40	14-27	18-24	13-25	5.0-7.9
Manavari	3	9.2-11.7	47-116	55-82	200-388	99-160	1.6-16.5	1.0-10.7	1-14	15-30	12-22	14-23	12-23	2.5-7.3
Mathuravellai	3	9.1-11.9	64-110	68-81	268-416	88-167	1.4-5.4	1.0-3.3	0-9	21-32	11-20	14-23	12-23	4.0-8.5
Peria cumbu	3	8.9-11.7	47-119	66-94	230-366	118-210	1.3-10.8	1.0-8.2	-5-14	16-27	11-27	14-27	11-25	3.1-8.4
Podi cumbu	3	9.3-11.3	65-105	56-77	276-392	97-170	1.4-7.2	1.0-3.4	-4-10	19-37	12-26	16-27	12-20	4.5-6.9
Umi	3	9.3-10.8	92-115	71-79	323-359	100-180	1.0-4.4	1.0-3.2	2-6	18-33	13-19	14-23	13-17	4.2-6.8
Adchi	4	16.9-21.4	44-61	60-71	190-260	130-180	2.7-7.5	2.1-4.5	7-16	14-24	12-18	20-28	19-25	8.0-10.0
Amreli	4	21.6	47-56	55-76	149-250	135-170	1.3-3.3	1.0-3.3	-8-10	23-35	16-28	22-33	22-25	7.1-8.9
Arisi	4	9.6-11.5	48-100	56-90	176-360	87-200	2.0-8.9	1.0-6.8	-3-14	16-27	12-24	15-28	11-24	2.5-9.0
Babapuri	4	19.4-21.9	43-66	57-68	151-270	130-200	1.2-4.3	1.0-3.3	3-10	17-28	16-24	20-35	20-30	7.0-9.9
Gaorani	4	17.1-21.8	46-67	45-79	146-270	110-210	1.0-6.5	1.0-4.6	-6-15	12-30	11-23	19-29	18-31	6.4-10.7
Kattu cumbu	4	9.4-11.5	48-119	61-94	175-380	108-335	1.0-18.5	1.0-12.5	0-14	17-31	12-25	13-24	11-25	2.0-8.5
Kullan	4	9.3-12.9	47-119	57-95	180-346	106-180	1.0-12	1.0-9.4	-9-14	16-35	11-30	15-25	12-23	3.3-8.3
Mainpuri	4	25.9-26.8	52-64	56-74	239-300	143-187	1.4-3.2	1.4-2.5	1-8	25-28	23-27	21-26	20-27	6.5-8.8
Nattu cumbu	4	9.3-12.4	51-98	59-76	190-384	92-200	1.8-7.4	1.0-4.4	-8-11	18-35	12-21	12-27	11-27	3.0-9.5
Oomi cumbu	4	9.4-9.5	71-100	61-77	242-366	60-170	1.8-4.5	1.0-3.6	4-13	15-30	10-28	15-24	10-20	2.5-7.0
Pedda sajja	4	14.4-18.8	46-86	55-74	160-370	135-250	1.8-5.5	1.3-4.3	6-11	18-30	14-27	20-30	16-27	5.5-8.7
Pokku cumbu	4	9.9-11.3	54-89	53-76	170-250	68-195	2.6-10.8	2.1-10.6	3-17	15-25	8-22	13-17	10-18	3.7-4.8
Pottu cumbu	4	10.4-12.5	47-105	60-74	208-310	136-185	1.8-6.5	1.8-4.3	5-11	19-30	13-21	13-30	11-25	4.0-8.0
Sadguru bajra	4	18.3-19.7	45-99	61-77	180-270	150-180	2.0-5.2	1.5-3.8	-3-15	17-22	15-31	19-25	16-25	6.0-10.0
Vellai	4	9.3-11.7	47-98	56-82	204-264	72-174	2.4-8.5	2.2-5.0	6-15	14-32	10-17	15-23	12-25	4.0-6.1

Table 4. Mean values for different traits of pearl millet named landraces from India, evaluated at ICRISAT, India

Landrace	Cl. no.	Days to 50% flowering-R	Days to 50% flowering-PR	Plant height (cm)-R	Plant height (cm)-PR	Total tillers (no)	Productive tillers (no)	Panicle exsertion (cm)	Panicle length (cm)-R	Panicle length (cm)-PR	Panicle thickness (mm)-R	Panicle thickness (mm)-PR	1000-Seed weight (g)
Bchwaddawana	1	50.3	67.9	184.0	167.5	1.9	1.6	4.4	24.6	21.4	20.5	18.1	6.6
Chadi local	1	55.9	65.4	182.9	184.8	5.2	3.8	8.4	21.3	15.7	18.5	17.6	7.4
Desert type	1	57.1	67.1	174.3	191.3	5.2	3.7	8.6	20.1	14.8	16.4	16.1	7.0
Gullisita	1	61.8	68.9	227.6	188.9	2.8	2.5	3.5	25.6	20.5	23.8	21.6	7.4
Mahudo	1	61.0	65.2	174.8	195.8	2.0	1.7	4.7	21.2	20.2	21.8	23.5	8.0
Pitta ganti	1	57.0	69.7	205.2	181.6	4.3	2.9	6.9	19.9	16.4	20.1	18.6	5.6
Barmer local	2	60.5	70.5	206.4	195.9	3.5	2.9	8.7	27.4	19.8	18.2	18.8	7.3
Jakhrana	2	50.8	66.5	200.0	165.0	2.5	2.3	6.8	30.6	26.1	20.9	20.3	7.8
Karauli	2	62.5	71.4	246.3	215.9	2.7	2.4	5.2	31.3	23.9	19.9	20.2	7.9
Sulkhania	2	61.7	70.1	240.0	197.9	2.6	2.5	7.6	33.3	23.9	17.3	18.6	7.4
Eravai	3	100.9	74.6	331.9	129.4	2.6	1.5	3.3	27.9	17.3	18.8	14.8	5.4
Kala cumbu	3	81.7	78.7	259.2	151.7	3.4	2.3	4.2	27.3	20.0	21.0	17.3	6.0
Manavari	3	91.4	71.8	327.2	137.3	3.3	2.1	5.2	25.1	16.2	18.7	15.3	5.5
Mathuravellai	3	97.5	72.8	347.2	137.2	2.8	1.6	3.4	26.0	16.0	18.5	14.4	5.7
Peria cumbu	3	87.2	78.3	300.3	159.6	4.9	3.0	7.0	21.5	17.1	17.7	15.7	4.8
Podi cumbu	3	91.5	70.7	342.7	145.4	2.8	1.7	4.9	27.2	17.9	20.4	15.5	5.8
Umi	3	102.2	74.6	347.4	137.6	2.8	1.8	4.0	27.0	16.2	19.2	14.6	6.0
Adchi	4	51.3	66.1	220.0	160.0	4.9	3.3	10.0	18.7	15.1	24.7	21.9	9.0
Amreli	4	52.0	67.2	202.8	150.6	2.6	2.1	4.0	27.6	21.2	26.0	23.4	8.2
Arisi	4	70.8	71.4	250.2	144.4	4.7	3.4	7.1	20.9	16.2	19.4	16.9	5.5
Babapuri	4	53.0	63.6	196.4	166.7	2.7	2.1	6.3	23.4	20.0	26.1	25.7	8.6
Gaorani	4	57.8	62.2	194.2	155.5	2.8	2.0	6.9	18.1	15.9	23.9	24.0	8.5
Kattu cumbu	4	79.3	72.6	291.2	155.5	7.9	5.3	9.1	21.8	18.6	17.8	17.7	5.0
Kullan	4	65.2	71.8	226.6	140.4	4.1	3.2	8.2	20.7	18.1	19.7	16.1	5.7
Mainpuri	4	59.6	62.6	266.2	164.0	2.2	1.9	3.8	26.2	24.0	23.6	22.0	7.8
Nattu cumbu	4	74.3	66.2	284.8	145.3	3.5	2.5	7.6	24.1	17.4	20.7	18.5	5.9
Oomi cumbu	4	86.6	69.4	312.2	118.5	3.2	2.4	8.0	24.4	16.0	19.1	14.8	5.5
Pedda sajja	4	56.9	62.9	225.3	168.5	3.4	2.5	8.6	23.5	20.2	23.6	21.5	7.3
Pokku cumbu	4	67.6	66.7	198.8	117.3	5.5	5.0	8.9	17.8	13.6	15.3	13.1	4.1
Pottu cumbu	4	66.9	68.7	242.6	157.9	3.9	3.0	8.6	23.7	17.3	21.4	19.1	6.2
Sadguru bajra	4	67.1	65.4	233.3	163.9	3.4	2.5	7.9	19.7	18.7	22.4	21.7	7.5
Vellai	4	73.0	67.5	236.7	119.2	4.5	3.5	9.7	21.3	13.5	20.0	15.2	5.3

less and produced many total as well as productive tillers during rainy season; those of cluster 2 produced long panicles during both the seasons and produced stout panicles and larger seeds during postrainy season; those of cluster 3 grew less during postrainy season and those of cluster 4 produced many total as well as productive tillers and highly exserted stout panicles during rainy season. Cluster 2 during postrainy season and cluster 3 during rainy season were good sources for tall accessions.

Mean values for individual landraces indicated that all landraces from lower latitudes (9^{o}N-13^{o}N) flowered late (>65 days) during rainy and postrainy season and grew tall (>250 cm) during rainy season and relatively short (<160 cm) during postrainy season and produced small and thin panicles (Table 4).

***Variances*:** The homogeneity of variances for different clusters and eight quantitative traits was tested by Levene's test (Levene, 1960). The variances were heterogeneous (p=0.001) for days to 50% flowering during both the seasons, plant height during rainy season, total and productive tillers, panicle length during rainy season, panicle thickness during both the seasons and 1000 seed weight, which was recorded during postrainy season (Table 2). Variance was more in cluster 4 for plant height and panicle exsertion during rainy season.

***Phenotypic diversity*:** Mean Shannon-Weaver diversity (H`) over all traits was maximum (H`=0.598±0.006) in cluster 2 (Table 5). Mean diversity over clusters indicated maximum diversity (H`=0.616±0.004) for 1000 seed weight. Among the clusters, cluster 1 for days to 50% flowering during postrainy season; cluster 2 for 1000 seed weight; cluster 3 for panicle exsertion and cluster 4 for panicle thickness during rainy season were highly diverse with maximum diversity index (H`).

Correlations

Twenty-four correlations were highly significant and positive, 29 were highly significant and negative, one significant and positive, and three significant and negative (Table 6). Days to 50% flowering during rainy season had highly significant positive correlation with days to 50% flowering during postrainy season, plant height and panicle length during rainy season. Days to 50% flowering during postrainy season also had highly significant positive association with plant height during rainy and postrainy seasons and panicle length during rainy season and highly significant negative correlation

Table 5. Shannon-Weaver (H`) diversity index for different traits of Indian pearl millet landraces in different clusters, evaluated at ICRISAT, India

Trait	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Mean	Se±
Days to 50% flowering-R	0.602	0.590	0.537	0.568	0.574	0.0142
Days to 50% flowering-PR	0.614	0.550	0.512	0.618	0.573	0.0259
Plant height (cm)-R	0.613	0.623	0.564	0.567	0.592	0.0152
Plant height (cm)-PR	0.591	0.608	0.609	0.606	0.604	0.0041
Total tillers (no.)	0.513	0.589	0.436	0.392	0.483	0.0434
Productive tillers (no.)	0.571	0.594	0.320	0.377	0.466	0.0687
Panicle exsertion (cm)	0.605	0.604	0.636	0.542	0.597	0.0199
Panicle length (cm)-R	0.573	0.600	0.622	0.582	0.594	0.0108
Panicle length (cm)-PR	0.536	0.585	0.593	0.630	0.586	0.0195
Panicle thickness (mm)-R	0.604	0.623	0.588	0.636	0.613	0.0107
Panicle thickness (mm)-PR	0.625	0.585	0.551	0.596	0.589	0.0153
1000-Seed weight (g)	0.609	0.624	0.610	0.621	0.616	0.0038
Mean	0.588	0.598	0.548	0.561	0.574	
Se±	0.0098	0.0061	0.0262	0.0252	0.0140	

R= Rainy and PR=Postrainy season

with panicle exsertion, panicle thickness during rainy and postrainy season, and 1000 seed weight. Strong positive correlation between days to 50% flowering and plant height during rainy season (r=0.811) explains more than 50% variation and suggests that selection for early flowering will result in selection of accessions with less height. Similarly, total tiller per plant showed strong positive correlation with productive tillers per plant (r=0.884) indicating the selection for more total tillers per plant results in selection for more productive tillers per plant. Selection for long panicles during rainy season results in selection for pearl millet producing long panicles during postrainy season indicating the less effect of season on panicle length. Differences in correlation coefficients of trait combinations during rainy and postrainy seasons may be attributed to the effect of photoperiod and temperature sensitivity of the accessions.

Climate

A clear pattern regarding association of latitude of collection sites, climatic factors and adaptation of named landraces was observed in the collection under study (Fig. 2). As expected, the highest monthly mean day length and maximum temperature at collection sites of landraces originated at higher latitudes (cluster 1 and cluster 2 and Mainpuri, Amreli, Babapuri, Sadguru bajra, Gaorani and Adchi of cluster 4) was relatively higher than all landraces of cluster 3 and remaining landraces of cluster 4 and it increased with the increase in latitude. Highest monthly mean minimum temperature also followed the pattern of latitude. On the other hand, lowest monthly mean minimum temperature deviated from the pattern of latitude and reduced with the increase in latitude of collection sites. All landraces, except Pitta ganti, in cluster 1with mean latitude of more than 20^{o}N had received mean annual rainfall ranging between 294-422 mm. On the other hand, collection sites of Pitta ganti with latitude range of 14.5^{o}-19.3^{o}N had received mean annual rainfall of 1149 mm. Collection sites of landraces in cluster 2 received mean annual rainfall ranging between 390-562 mm, those of cluster 3 received 778-883 mm and those of cluster 4 received 573-954 mm.

Identification of Promising Sources

Promising sources were identified within each cluster for different agronomic and nutritional traits, photoperiod and temperature insensitivity, salinity and disease resistance (Table 7).

Agronomic traits

Of the 692 accessions under study, 203 accessions (29%) belonging to 29 landraces (90% landraces under study) originating in nine provinces, were identified as the promising sources for one or more traits. Among the provinces, accessions from Tamil Nadu were promising for fodder yield and those from Rajasthan, Andhra Pradesh and Maharashtra for early flowering (<50 days) during rainy season. Among the clusters,

Table 6. Correlation coefficients for different traits of Indian pearl millet landraces, evaluated at ICRISAT, India

Trait	1	2	3	4	5	6	7	8	9	10	11
2	0.478										
3	0.811	0.426									
4	-0.308	0.089	-0.215								
5	0.054	0.029	-0.006	-0.076							
6	-0.070	-0.015	-0.112	0.015	0.884						
7	-0.194	-0.186	-0.228	0.025	0.288	0.297					
8	0.088	0.144	0.293	0.263	-0.259	-0.214	-0.36				
9	-0.190	0.043	0.054	0.400	-0.184	-0.122	-0.221	0.615			
10	-0.276	-0.251	-0.107	0.065	-0.327	-0.312	-0.187	0.106	0.175		
11	-0.464	-0.309	-0.293	0.364	-0.180	-0.108	-0.07	0.124	0.418	0.527	
12	-0.477	-0.284	-0.377	0.389	-0.319	-0.247	-0.018	0.205	0.255	0.396	0.461

Correlations ≥0.088 are significant at p=0.01% and ≥0.062 are significant at p=0.05% at 690 df

1= Days to 50% flowering-R, 2 = Days to 50% flowering-PR, 3 = Plant height (cm)-R, 4 = Plant height (cm)-PR, 5 = Total tillers (no), 6 = Productive tillers (no), 7 = Panicle exsertion (cm), 8 = Panicle length (cm)-R, 9 = Panicle length (cm)-PR, 10 = Panicle thickness (mm)-R, 11 = Panicle thickness (mm)-PR, 12 = 1000-Seed weight (g)

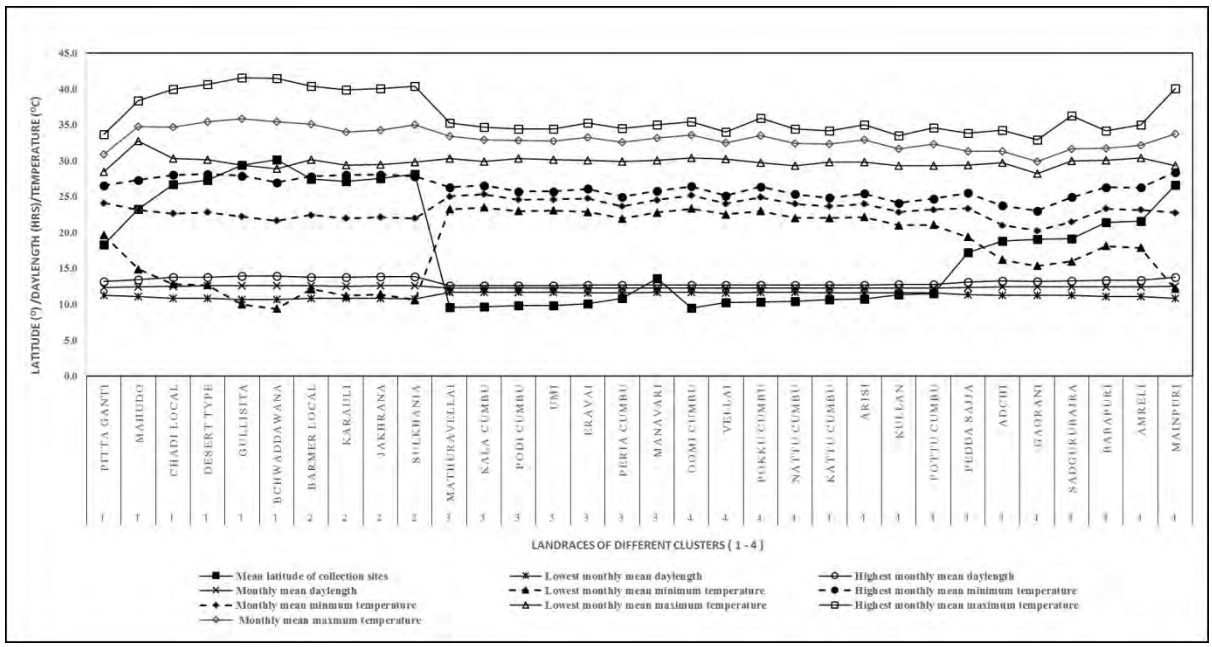

Fig. 2. Climatic factors at the collection sites of pearl millet named landraces from India, conserved at ICRISAT, India

Table 7. Promising landraces identified for different agronomic and nutritional traits and biotic and abiotic stresses, evaluated at ICRISAT, India

Trait	Cluster no.	Promising source : Landrace (IP no.)
Agronomic traits		
Days to 50% flowering - R (<50 days)	1	Bchwaddawana (IP 1417, 1418, 1425, 1427), Chadi local (3155, 3172, 3175, 3220, 3226, 3231, 3235, 3239, 3252, 3253, 3435, 13586), Desert type (3230, 3251), Pitta ganti (1249, 1251, 1252, 11782-11784, 11786, 11787, 11789-11792, 11794, 11867, 11868, 11873, 11877, 11878, 13460, 13465)
	2	Jakhrana (IP 1478-1480, 3123 and 11885),
	3	Kala cumbu (1315), Manavari (1304), Peria cumbu (1307)
	4	Adchi (9101, 9105, 17867), Arisi (15329 and 15412), Babapuri (1179, 3840, 12556), Gaorani (9107, 9113, 9115, 9121, 9122, 9146, 13554), Kattu cumbu (15335, 15373), Kullan (1306), Pedda sajja (11810, 11811), Sadguru bajra (1266, 1267, 7813) and Vellai (13600)
Days to 50% flowering - PR (<50 days)	4	Gaorani (4144, 4148 and 4149)
Plant height-R (<150 cm)	1	Bchwaddawana (1416, 1425), Desert type (3245 and 3264), Mahudo (3865, 3857), Pitta ganti (3018, 13465)
	4	Amreli (3807), Gaorani (4148, 4150)
Plant height-PR (<100 cm)	3	Manavari (3576), Mathuravellai (3582), Podi cumbu (3577)
	4	Arisi (3475), Nattu cumbu (3573), Oomi cumbu (3569, 3570 and 3572), Pokku cumbu (3583, 3589, 3590 and 3591), Vellai (3487 and 3574)
Plant height-R (>375 cm)	3	Eravai (3495), Manvari (3516 and 3554), Mathuravellai (3498, 3499, 3508, 3515, 3544), Podi cumbu (3525, 3529, 3532)
	4	Kattu cumbu (3568, 3597) and Nattu cumbu (3559)

Contd.

Traits	Cluster No.	Promising source : Landrace (IP no.)
Plant height-PR (250-350 cm)	1	Gullisita (3274)
	2	Karauli (3366, 3367, 3370, 3382)
	4	Kattu cumbu (16238)
Total tillers (15.5 - 18.5)	1	Chadi local (13584), Pitta ganti (3036)
	3	Manavari (15341)
	4	Kattu cumbu (3628, 15285, 15288, 15290, 15301, 15302, 15304, 15342 and 15351)
Panicle length-R (42-50 cm)	1	Gullisita (13575)
	2	Jakhrana (13581), Karauli (3338, 3349, 3393, 3395, 13582) and Sulkania (3316)
	4	Adchi (17877) and Gaorani (13552)
Green fodder yield potential (score 8)	1	Chadi local (3188, 3246), Desert type (3173 and 3190)
	3	Eravai (3517), Kala cumbu (3586), Peria cumbu (1305, 3610)
	4	Amreli (1174), Gaorani (9074 and 9076), Kattu cumbu (3479 and 3596) and Sadguru bajra (16283)
Seed yield potential (score 8)	1	Chadi local (3233, 3234), Desert type (3244, 3245)
	3	Peria cumbu (3477)
	4	Gaorani (9119)
Nutritional traits		
Sweet stalk	3	Podi cumbu (3471), Podi cumbu (3481), Mathuravellai (3509) and Umi (3593)
High seed protein (>15.0%)	3	Peria cumbu (3611, 3615, 3626), Podi cumbu (3481) and Mathuravellai (3620)
	4	Oomi cumbu (15400), Kullan (3531), Arisi (3631)
High seed iron and zinc (>50 ppm)	1	Chadi local (3432), Gullisita (11044), Mahudo (3859), Pitta ganti (11799)
	2	Karauli (3329, 3331)
	3	Podi cumbu (3525), Peria cumbu (3626)
	4	Kullan (3642), Pedda sajja (11811), Kattu cumbu (15273)
Biotic stresses		
Downy mildew resistance	1	Chadi local (3220), Gullisita (3274), Pitta ganti (3060)
	2	Karauli (3146, 3359, 3382)
	3	Manavari (3492), Mathuravellai (3509, 3526, 3582), Podi cumbu (3543, 13603), Umi (3486)
	4	Arisi (3480), Kattu cumbu (3479, 15273), Kullan (3484, 3531) and Mainpuri (13620)
Rust resistance	4	Kullan (3531), Pedda sajja (8521)
Abiotic stresses		
Heat tolerance	1	Chadi local (3175)
Salinity tolerance	3	Peria cumbu (3616)
Photoperiod and temperature insensitivity	1	Chadi local (3228, 3419)
	2	Barmer local (3304) and Karauli (3331)
	4	Arisi (3617), Mainpuri (6901) and Kattu cumbu (15433)

44.3% accessions of cluster 4, 30% of cluster 1, 23% of cluster 2 and 22% of cluster 3 were promising for one or more traits. A total of 64 accessions belonging to six landraces in cluster 1 for ten traits, 25 accessions of three landraces in cluster 2 for five traits, 25 accessions of six landraces in cluster 3 for seven traits and 89 accessions of 14 landraces in cluster 4 for 14 traits were superior over the other accessions. Among the landraces, Chadi local and Manavari for five traits, Gaorani and Kattu cumbu for seven traits, were identified as important sources. In the entire collection, range of variation indicated 71 accessions belonging to 18 landraces as promising for early flowering (<50 days) during rainy season (Table 7). Only three accessions of Gaorani (IP 4144 IP 4148 and IP 4149) were identified for early flowering (<50 days) during postrainy season. In the

collection, 11 accessions of six landraces for short plant height (<150 cm) during rainy season, 14 accessions of eight landraces for short height during postrainy season; 14 accessions of six landraces for tallness (>375 cm) during rainy season and six accessions of three landraces for tallness (250-350 cm) during postrainy season, were important sources (Table 7).

Drought tolerance

Generally, pearl millet grown in low rainfall dry regions is considered a good source ofdrought-tolerance. Annual mean rainfall at the collection sites of cluster 1 landraces (<425 mm) was low when compared with that of cluster 2, cluster 3 and cluster 4. Low annual mean rainfall averaged over all collection sites of Desert type accessions (294 mm) in cluster 1 indicated that they could be good sources for drought-tolerant pearl millet.

Photoperiod and temperature insensitivity

A maximum of 92.6% accessions in cluster 1, 94.7% in cluster 2, 55.4% in cluster 4 and 13.2% in cluster 3 had shown temperature sensitivity (Table 7). On the other hand, 86.8% accessions in cluster 3, 42.6% in cluster 4, 6.5% in cluster 1 and 4% in cluster 2 had shown photoperiod sensitivity. Two accessions (IP 3228 and 3419) of Chadi local; IP 3304 of Barmer local; IP 3617 of Arisi; IP 6901 of Mainpuri; IP 3331 of Karauli and IP 15433 of Kattu cumbu were promising sources for photoperiod and temperature insensitive pearl millet.

Nutritional traits

Pearl millet germplasm database for seed quality traits indicated the evaluation of limited number of accessions under study for these traits. One accession each of Chadi local, Gullisita, Mahudo, Pitta ganti, Karauli, Podi cumbu, Peria cumbu, Kullan, Pedda sajja and Kattu cumbu were promising sources with more than 50 ppm seed iron and zinc (Table 7). Peria cumbu, Podi cumbu, Mathuravellai, Oomi cumbu, Kullan and Arisi were important sources for high seed protein content (>15%) (Table 7). IP 3471, IP 3481, IP 3509 and IP 3593 were sweet stalks with high stalk soluble sugar content (17-19%).

Diseases resistance

Pearl millet germplasm database for disease reaction of accessions indicated that at least one accession each of Chadi local, Gullisita, Pitta ganti, Karauli, Manavari, Mathuravellai, Podi cumbu, Umi, Arisi, Kattu cumbu, Kullan, Mainpuri for downy mildew and Kullan and Pedda sajja for rust were the sources of resistance for downy mildew and rust with <5% disease severity (Table 7).

Multiple traits

IP 13465 of Pitta ganti was found promising for early flowering (38 days to 50% flowering) and short height (<150 cm) during rainy season (Table 7). Eight accessions of Kattu cumbu (IP Nos. 15273, 15285, 15288, 15290, 15301, 15302, 15342 and 15351) were found as promising for more productive as well as total tillers. IP 15273 was also found promising for high seed iron and zinc content (>50 ppm) and downy mildew resistance. IP 3531 of Kullan for high seed protein content, downy mildew and rust resistance; IP 3479 of Kattu cumbu for high fodder as well as seed yield potential with downy mildew resistance, were the other promising sources.

Discussion

Named landraces, evolved from conscious selection over decades by farmers for desirable traits, were considered as time tested cultivars. In India, many farmers still use landraces and often retain them even when they experiment with and adopt modern varieties. High adaptation to varying agroclimatic conditions, high fodder yield, yield stability, resistance to abiotic and biotic stresses, perception of good quality food, nutritional and processing qualities, traditional intercropping patterns, religious ceremonies and filling unique market niches are the main reasons for liking of landraces by farmers. Because of open pollination in farmer's fields, landraces are heterogeneous populations and farmers grow such landraces either as a sole crop or mix with improved cultivars with a strong belief that they will harvest something from landraces even if they lose the crop of improved cultivars under adverse conditions (Jarvis *et al*., 2008). Smithson and Lenne (1996) reported the reduction in pest and disease attack, and higher yield stability due to poly-cultivar cropping. There is a need to foster diversity in food production and consumption, and thus improved food and nutritional security (Khourya *et al*., 2014). Therefore, adoption of wide range of cultivars or landraces is emphasized

worldwide, to boost the genetic diversity and thus reduce the genetic vulnerability of the world food system in the face of challenges including climate change and rising food demand.

The landraces under study were adapted to a wide range of latitudes (8.8^{o}N to 30.9^{o}N) and these were cultivated in more than one province. Variation in latitude of collections will have the impact on diversity among landraces. The close association of latitude and climatic conditions at collection sites and agronomic performance of landraces under study indicates no effect of geographic province (Upadhyaya *et al*., 2007; Upadhyaya *et al*., 2014). In the present study, landraces of cluster 1, except Pitta ganti, and those of cluster 2 were adapted to higher latitudes (14.53^{o}N to 30.90^{o}N) characterized by longer days, higher mean maximum temperature and low mean annual rainfall (<425 mm). These flowered relatively earlier (mean 57-61days) than those from lower latitudes (mean latitude <13^{o}N). These landraces may be useful in breeding early-medium maturing drought/temperature-tolerant varieties of pearl millet (Fig. 2) (Upadhyaya *et al*., 2014). All landraces of cluster 3 and Arisi, Kattu cumbu, Kullan, Nattu cumbu, Oomi cumbu, Pokku cumbu, Pottu cumbu, and Vellai of cluster 4 were from lower latitudes, characterized by day length close to 12 h and lower monthly mean minimum and maximum temperature. Probably due to sensitivity of the accessions to the climate, these landraces flowered late (mean 68-93days) and grew relatively tall during rainy season and short during postrainy season. At higher latitudes, lowest monthly mean minimum temperature was inversely proportion to the latitude of the collection site and was an important determinant of adaptation and diversity pattern (Upadhyaya *et al*., 2014). According to Upadhyaya *et al*. (2014), there was wide variation in pearl millet landraces from lower latitudes.

Accessions of Desert type, collected from very low rainfall (<300 mm) areas reflect the naming of landraces by farmers based on suitability of the material to deserts. Continuous selection over decades in dry regions might have resulted in Desert type landraces. Such landraces could be a good source for drought and heat tolerance and suitable for cultivation in drier regions of India. Upadhyaya *et al*. (2007) reported that the arid zone in India, characterized by low rainfall (<500 mm) and high temperatures (>35^{o}C), could be a better site for growing early maturing, short and high tillering pearl millet. However, number of rainy days and the amount of rainfall play an important role in deciding the length of growing period in any region (Gadgil *et al*., 1988). Yadav *et al*. (2003) reported local landraces as a good source of adaptive genetic variation for tolerance to drought and represent suitable breeding material for arid zone environments.

Accessions with high seed zinc content identified in the study could be useful in developing nutrient dense pearl millet varieties to reduce the problem of malnutrition in India. Sweet stalk accessions identified would provide good quality fodder (Appa Rao *et al*., 1982). Downy mildew and rust are the important diseases of pearl millet causing severe crop losses. Although pearl millet hybrids often give better grain yield than do the landraces, they are vulnerable to biotic and abiotic stresses. In such situations, the landrace germplasm identified as promising for downy mildew and rust are very useful in developing downy mildew- and rust-resistant pearl millet varieties and hybrids.

Because of continuous threat to the on-farm landraces due to their replacement by modern cultivars, natural calamities, urbanization, etc., specific measures are needed to safeguard them for future sustainable agriculture and food security (Upadhyaya and Gowda, 2009). Such measures include filling gaps in germplasm collections, documentation of landraces for indigenous knowledge, increasing awareness about role of landraces in sustainable agriculture and food security, establishing community genebanks conserving seeds of landraces for easy access to the smallholder farmers. Representation of India in world collection of pearl millet germplasm is impressive with 6,610 accessions. However, Upadhyaya *et al*. (2010) reported 134 districts of 14 provinces in India as geographical gaps and 208 districts of 12 provinces as gaps in trait-specific diversity. The collection sites of promising sources, identified in the present study, may be considered while launching collection missions to have more promising sources in the world collection. Tamil Nadu province, which was a good source with 15 out of 32 named landraces under study, may be further explored to enrich the diversity in fodder type pearl millet collection at ICRISAT. Upadhyaya *et al*. (2007) also recommended launching

germplasm collection programme in Tamil Nadu for high tillering pearl millet.

The promising sources identified in the present study are useful to broaden the genetic base of new cultivars and enhance the use of available germplasm resources. However, further evaluation of these sources for agronomic, nutritional and climate-related traits, and also for biotic stress resistance is needed for immediate release as varieties and also for long-term use in pearl millet improvement programmes. Seeds of all landrace accessions named in present study are available at ICRISAT genebank, India, for research and training purposes, under Standard Material Transfer Agreement (SMTA) of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA).

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Population Structure and Genetic Variation in Indian and African *Eleusine coracana* (L.) Gaertn

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Population structure and genetic variation was estimated for 40 Indian and African finger millet genotypes using 237 random amplified polymorphic DNA (RAPD) and 16 morphological markers revealing differences between African and Indian groups. African genotypes showed 53.59%; 0.17±0.19; 0.27±0.27 compared to 48.10%; 0.15±0.19; 0.24±0.27 polymorphism, Nei's gene diversity and Shannon's Information index, respectively in Indian genotypes. Primers OPM-20, OPW-04, OPX-11 were identified as the most informative primers in terms of high % polymorphism and Nei's gene diversity values. Population structure analysis revealed the existence of two distinct sub-groups, correlated well with primary and secondary centers of finger millet diversity and can be utilized to enhance and widen finger millet germplasm base.

Key Words: Finger millet, RAPD, Genetic diversity, Population structure

Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn., family Poaceae (Gramineae), subfamily Chloridoideae and tribe Eragrostideae; 2n=4x=36; genomic constitution AABB) is a subsistence food crop with excellent storage qualities and has high levels of calcium, iron, methionine-amino acid rich protein, soluble fibre and high diastatic power of malted grains (Barbeau and Hilu, 1993; Yenagi *et al*., 2010). Cultivated finger millet was domesticated some 5000 years ago in African highlands and moved to Indian subcontinent around 3000 B.C. There is vast scope for improving cultivars by intercrossing of African with Indian germplasm leading to good value addition to this crop. Evaluation of genotypic variation of Indian and African germplasm based on molecular markers along with its phenotypic characterization would play a vital role in selecting diverse parents for finger millet breeding programmes. Reports available on assessment of genetic diversity/population structure among finger millet genotypes at molecular level till date pertains to RAPD, SSR, cytochrome P450 gene based marker systems (Fakrudin *et al*., 2004; Babu *et al*., 2007; Dida *et al*., 2008; Panwar *et al*., 2010; Arya *et al.*, 2013). The objective of this study was to characterize a set of 40 finger millet genotypes originating from seven African countries and seven different states of India based on morphological and RAPD (RAPDs are valuable in terms of their high genomic abundance and random distribution throughout the genome) markers. Further, population sub-structure and partitioning of genetic variation was discussed in order to emphasize the utility of studied material in finger millet crop improvement.

Materials and Methods

Plant Materials

For the current study, the seeds of 40 diverse finger millet genotypes (Table 1) were procured from All India Coordinated Small Millet Improvement Project (AICSMIP), Bangalore, India.

Evaluation of Phenotypic Diversity

The selected finger millet genotypes were evaluated in Augmented Block Design for their agronomic performance at AICSMIP, Bangalore, India**.** Data were recorded for 16 morphological traits (Table 2).

DNA Extraction and PCR Amplification

Extraction of total genomic DNA was carried out following cetyl-trimethyl-ammonium bromide (CTAB) method (Saghai-Maroof *et al*., 1984) with minor modifications. DNA was extracted from bulk leaf samples (30 individual plants/sample) of 6-week-old

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Table 1. Forty finger millet genotypes used in the study and their sub-population membership coefficient

GE No.	Origin	G1	G2
GE28	Tamil Nadu, India	0.052	0.948
GE29	Tamil Nadu, India	0.026	0.974
GE62	Tamil Nadu, India	0.161	0.839
GE202	Tamil Nadu, India	0.075	0.925
GE595	Tamil Nadu, India	0.018	0.982
GE753	Tamil Nadu, India	0.017	0.983
GE1736	Tamil Nadu, India	0.010	0.990
GE1801	Tamil Nadu, India	0.010	0.990
GE174	Andhra Pradesh, India	0.053	0.947
GE314	Andhra Pradesh, India	0.022	0.978
GE617	Andhra Pradesh, India	0.017	0.983
GE1361	Andhra Pradesh, India	0.013	0.987
GE918	Karnataka, India	0.047	0.953
GE580	Bihar, India	0.022	0.978
GE1360	Andhra Pradesh, India	0.008	0.992
GE68	Uttaranchal, India	0.088	0.912
GE894	Uttaranchal, India	0.014	0.986
GE1924	Uttaranchal, India	0.031	0.969
GE565	Madhya Pradesh, India	0.026	0.974
GE594	Madhya Pradesh, India	0.011	0.989
GE468	Kerala, India	0.021	0.979
GE4690	Uganda, Africa	0.251	0.749
GE4682	Uganda, Africa	0.210	0.790
GE4725	Kenya, Africa	0.647	0.353
GE4752	Kenya, Africa	0.947	0.053
GE4765	Kenya, Africa	0.607	0.393
GE4796	Kenya, Africa	0.687	0.313
GE5121	Kenya, Africa	0.182	0.818
GE5130	Kenya, Africa	0.978	0.022
GE4915	Zambia, Africa	0.983	0.017
GE4927	Zambia, Africa	0.973	0.027
GE4951	Zambia, Africa	0.146	0.854
GE5025	Zimbabwe, Africa	0.972	0.028
GE5027	Zimbabwe, Africa	0.043	0.957
GE5029	Zimbabwe, Africa	0.976	0.024
GE4990	Tanzania, Africa	0.115	0.885
GE4991	Tanzania, Africa	0.982	0.018
GE4813	Malawi, Africa	0.980	0.020
GE4833	Malawi, Africa	0.989	0.011
GE5013	Ethiopia, Africa	0.986	0.014

plant. DNA quantification was done using DyNA Quant 200 fluorometer (Hoefer Instruments, USA). A total of twenty five primers with well resolved and distinct banding pattern were used for RAPD amplification. For the PCR reaction, a final volume of 25 µl was prepared. PCR amplification was carried out with 25 ng of genomic DNA, 3 mM $MgCl_2$ (Fermentas Life Sciences), 1U Taq DNA polymerase (Fermentas Life Sciences), 1x PCR buffer without $MgCl_2$ (Fermentas Life Sciences), 0.2 µM decamer primers (Operon) and 0.2 mM of dNTP mix (Fermentas Life Sciences). PCR reactions were carried out in a Perkin Elmer GeneAmp PCR system 9600 thermocycler. PCR reaction cycles consisted of initial denaturation at 94°C for 5 min, followed by forty cycles of denaturation at 94°C for 1 min, primer annealing at 37°C for 1 min and primer extension at 72°C for 2 min and final extension step at 72°C for 10 min. The amplification products were loaded onto a 1.4% agarose gel and were recorded using a Bio Imaging System (SynGene).

Statistical Analyses

The mean, range and standard deviation for each trait were calculated in order to identify which traits varied significantly between and within Indian and African subpopulations. RAPD fragments were scored visually, as absent (0) or present (1) and Rp (Resolving power) index was calculated as given by Prevost and Wilkinson (1999). POPGENE version 1.32 (Yeh *et al*., 2000) was used to analyze gene diversity and population differentiation. Jaccard's similarity coefficient was calculated for UPGMA clustering using NTSYS-pc. ver. 2.1 (Rohlf, 2000). In addition, the software STRUCTURE (Pritchard *et al*., 2000) was used to investigate population structure using a burn-in of 100,000, a run length of 10,00,000 (admixture model). The number of sub-groups/ populations (K) was determined by running the program at different K values (1 to 6). Five independent runs were assessed for each K value. Number of distinct sub-populations was finally inferred based on maximum Ln P(D) and peak value of delta K (Evanno *et al*., 2005).

Results and Discussion

Average plant height, culm thickness, productive tillers, leaf number, flag leaf blade length, peduncle length, finger length, days to 50% flowering, days to maturity and grain yield (g) were higher across African as compared to Indian group (Table 2). Similar observations were also recorded by earlier workers (Dida *et al*., 2008).

Table 2. Mean, Range and Standard Deviation (SD) for the phenotypic traits analyzed

Trait	African genotypes			Indian genotypes		
	Mean	Range	SD	Mean	Range	SD
Plant height (cm)	98.25	58-137	21.84	94.86	61-117	14.93
Culm thickness (cm)	1.01	0.8-1.3	0.13	0.82	0.6-1	0.11
Productive tillers	4.78	3.4-7.2	1.04	4.19	3.0-7	1.29
Leaf numbers	10.90	8.0-15	1.92	9.14	7.0-11	1.28
Flag leaf blade length (cm)	34.12	21.4-48.6	8.87	30.55	21.1-45.1	6.86
Flag leaf leaf width (cm)	1.07	0.9-1.5	0.17	0.97	0.6-1.2	0.13
Leaf length (cm)	38.87	25.8-55.6	10.27	44.53	30-52.4	5.48
Leaf width (cm)	1.18	1.02-1.5	0.14	1.19	0.9-1.5	0.17
Peduncle length (cm)	24.56	19-30.8	3.48	24.00	18.0-32	3.77
Finger number	6.84	5.6-8.8	1.01	7.24	5.0-12	1.61
Finger length (cm)	6.83	4.7-9.8	1.38	5.95	3.7-8.8	1.48
Finger width (cm)	1.12	1.02-1.3	0.08	0.96	0.8-1.2	0.11
Days to 50 per cent flowering	68.75	63-73	3.49	58.00	46-68	6.43
Days to maturity	114.75	99-125	8.03	98.52	86-113	7.89
Test weight (g)	2.04	1.9-2.4	0.14	2.17	1.1-3.6	0.55
Grain yield (g)	20.01	9.1-33.0	6.50	10.48	2.0-19.0	4.61

Leaf length, finger number and test weight (g) was more for Indian as compared to African group in the tested location. Region wise data for these traits are not shown here. Mean values for leaf length and test weight was highest for genotypes from Tamil Nadu. Mean finger number was highest for genotypes from Uttaranchal. Mean culm thickness was highest in Kenyan genotypes. Genotypes from Malawi and Zambia showed longest mean value of days to 50% flowering and days to maturity respectively. Mean grain yield was highest for Zambian genotypes. And for rest of the traits Ugandan genotypes were showing highest mean values.

A total of 237 RAPD bands were amplified using 25 primers with a mean of 9.48 bands per primer, which was comparable to earlier studies (Babu *et al.* 2007, Panwar *et al.* 2010). Of the 237 RAPD fragments, 155 (65.4%) were polymorphic and the average number of polymorphic bands per primer was 6.2. The resolving power for the primer OPM-20 was 6.5 and was most informative followed by OPV-06 (4.61) and OPL-07 (4.35) in distinguishing individual genotypes. For twenty one Indian genotypes, a total of 229 bands were generated and 114 of them were polymorphic (48.10%) while African genotypes amplified 127 polymorphic (53.59%) out of 230 bands (Table 3). Our results indicated that African genotypes were showing relatively more polymorphism as compared to Indian genotypes, similar to previous reports (Fakrudin *et al*., 2004; Dida *et al*., 2008; Arya *et al*., 2013).

The RAPD primers varied in their power to detect diversity within Indian and African genotypes. Some primers, such as OPM-20, OPW-04, OPX-11, revealed high diversity levels in both the African and Indian genotypes, whereas OPC-06 and OPH-12 detected very low variation across the two groups. The mean diversity value for all the 40 genotypes was 0.18±0.18 and Shannon's Information index ranged from 0.09 to 0.48 with a mean of 0.29±0.25. Averaged over all the markers and genotypes, African genotypes displayed higher genetic variation (0.17±0.19) as compared to Indian population (0.15±0.19) and also higher mean Shannon's Information index for African (0.27±0.27) as compared to Indian sub-population (0.24±0.27). Lower diversity within Indian population as compared to African genotypes suggested that Indian sub-population arose from a relatively small number of founder plants (Dida *et al*., 2008).

Region wise, genetic diversity parameters like percent of polymorphic loci, the mean observed number of alleles, the mean effective number of alleles, the mean Nei's gene diversity and mean Shannon's Information index are shown in Table 4. The results revealed that finger millet genotypes from Tamil Nadu region of the Indian subcontinent were most divergent as compared to other regions. In case of African sub-population, genotypes from Zambia followed by Kenya were most

Table 3. Comparison of the level of polymorphism and diversity between Indian and African genotypes using RAPD markers

	Indian genotypes					African genotypes				
Primer	TNB	NPB	P (%)	h	I	TNB	NPB	P (%)	h	I
OPB-07	9	4	44.44	0.17	0.26	9	1	11.10	0.03	0.05
OPB-08	8	2	25.00	0.06	0.10	9	4	44.44	0.09	0.16
OPB-18	14	10	71.40	0.21	0.32	13	9	69.20	0.19	0.29
OPC-06	5	1	20.00	0.07	0.11	5	1	20.00	0.03	0.05
OPD-08	14	7	50.00	0.19	0.28	14	7	50.00	0.16	0.25
OPD-13	6	4	66.66	0.14	0.23	6	3	50.00	0.14	0.21
OPF-14	5	2	40.00	0.13	0.20	5	4	80.00	0.33	0.49
OPF-20	8	4	50.00	0.14	0.22	8	3	37.50	0.15	0.23
OPG-02	8	5	62.50	0.18	0.28	8	6	75.00	0.27	0.40
OPH-12	10	4	40.00	0.07	0.11	9	2	22.20	0.04	0.06
OPH-19	8	3	37.50	0.12	0.18	9	6	66.66	0.27	0.40
OPL-01	8	3	37.50	0.15	0.22	8	4	50.00	0.16	0.24
OPL-07	15	11	73.30	0.23	0.34	14	10	71.40	0.19	0.30
OPM-05	11	3	27.20	0.07	0.11	11	6	54.50	0.20	0.30
OPM-07	9	4	44.44	0.15	0.23	9	6	66.66	0.18	0.29
OPM-18	8	4	50.00	0.13	0.20	9	5	55.50	0.16	0.25
OPM-20	14	12	85.75	0.31	0.46	14	11	78.50	0.29	0.42
OPV-06	13	7	53.80	0.21	0.31	12	8	66.66	0.23	0.34
OPV-07	12	3	25.00	0.08	0.12	14	5	35.70	0.13	0.19
OPW-04	9	6	66.66	0.23	0.35	9	8	88.80	0.22	0.36
OPW-19	7	2	28.50	0.09	0.14	7	3	42.80	0.17	0.25
OPW-20	7	3	42.80	0.12	0.19	7	3	42.80	0.19	0.27
OPX-11	5	4	80.00	0.23	0.37	5	4	80.00	0.23	0.36
OPX-14	10	3	30.00	0.09	0.14	10	3	30.00	0.08	0.13
OPX-17	6	3	50.00	0.10	0.17	6	5	83.30	0.23	0.38
	229	114	48.10	0.15	0.24	230	127	53.59	0.17	0.27

TNB= Total number of bands; NPB= Number of polymorphic bands; P(%)= % Polymorphism; h= Nei's gene diversity; I= Shannon's information index

Table 4. Genetic diversity parameters of genotypes collected from diverse regions of India and Africa

Region	% (P)	na	ne	h	I
Tamil Nadu	35.86	1.3586±0.4806	1.2626±0.3890	0.1449±0.2068	0.2101±0.2940
Andhra Pradesh	26.16	1.2616±0.4404	1.1924±0.3570	0.1057±0.1879	0.1535±0.2675
Uttaranchal	16.46	1.1646±0.3716	1.1214±0.2936	0.0676±0.1572	0.0983±0.2257
Madhya Pradesh	10.13	1.1013±0.3023	1.0716±0.2138	0.0419±0.1252	0.0612±0.1828
Uganda	13.08	1.1308±0.3379	1.0925±0.2389	0.0542±0.1400	0.0791±0.2043
Kenya	27.85	1.2785±0.4492	1.2172±0.3731	0.1183±0.1979	0.1701±0.2810
Zambia	32.49	1.3249±0.4693	1.2476±0.3858	0.1363±0.2036	0.1971±0.2903
Zimbabwe	22.36	1.2236±0.4176	1.1777±0.3509	0.0964±0.1846	0.1385±0.2624
Tanzania	19.83	1.1983±0.3996	1.1402±0.2825	0.0821±0.1655	0.1199±0.2416
Malawi	10.55	1.1055±0.3078	1.0746±0.2177	0.0437±0.1275	0.0638±0.1862

na= Observed number of alleles; ne= Effective number of alleles; h= Nei's gene diversity; I= Shannon's information index (Sample size was one for Karnataka, Bihar, Kerala and Ethiopia regions and hence not included for this analysis)

divergent. And genotypes from Madhya Pradesh and Malawi showed the lowest genetic variation. Greater genetic gains can be obtained by using these genotypes in finger millet crop improvement.

The Jaccard's similarity coefficients (JSC) values for RAPD markers ranged from 0.68 to 0.93 with a mean JSC value of 0.82. The present study identified GE 4927 (Zambia) and GE 62 (Tamil Nadu) as diverse genotypes, which were also found distinct on the basis of morphological traits like plant height (67.4 cm, 112 cm), productive tillers (4.2, 6), leaf length (31.6 cm, 50 cm), finger width (1.12 cm, 0.9 cm), peduncle length (19 cm, 32 cm), days to 50% flowering (71, 59) and days to maturity (119, 95) respectively. The mean value of JSC for the RAPD markers for Indian genotypes is 0.84 and 0.82 for African genotypes, thereby showing more variation in African as compared to Indian genotypes. The Jaccard's similarity based dendrogram was also constructed for the RAPD markers and is depicted in Fig. 1. The forty genotypes were divided into two groups. Group I consists of five sub-groups (IA, IB, IC, ID and IE) that includes all the 21 Indian and 11 African (8 are in sub-group IC) genotypes. Group II consists of seven African genotypes only. GE 4927 from Zambia is present as an outlier. Most of these genotypes were clustered by geography. For example, the Indian and African genotypes were grouped in separate clusters, which may be due to the presence of distinct morphological characters between two sub-populations. Intermixing of African genotypes in Indian cluster was also observed, which suggested that Indian germplasm appears to be derived from African introductions. This also supports the previous report (Dida *et al*., 2008; Arya *et al*., 2013) that finger millet was first domesticated in Africa and then spread to India.

The results of the STRUCTURE analysis (Fig. 2) based on maximum Ln P(D) and peak value of delta K inferred the number of populations, K to be 2. Of the 40 finger millet genotypes, 34 (85.0%) shared > 70% membership with one of two sub-groups (G1 and

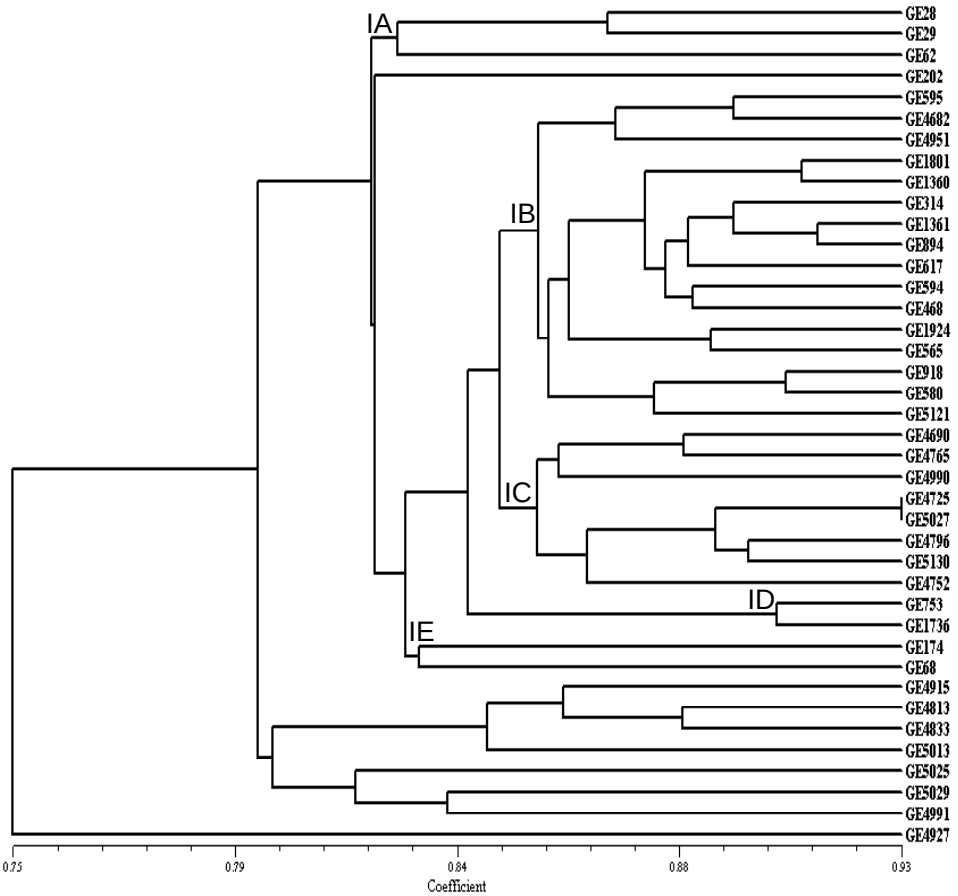

Fig. 1. UPGMA dendrogram based on RAPD marker data of 40 finger millet genotypes

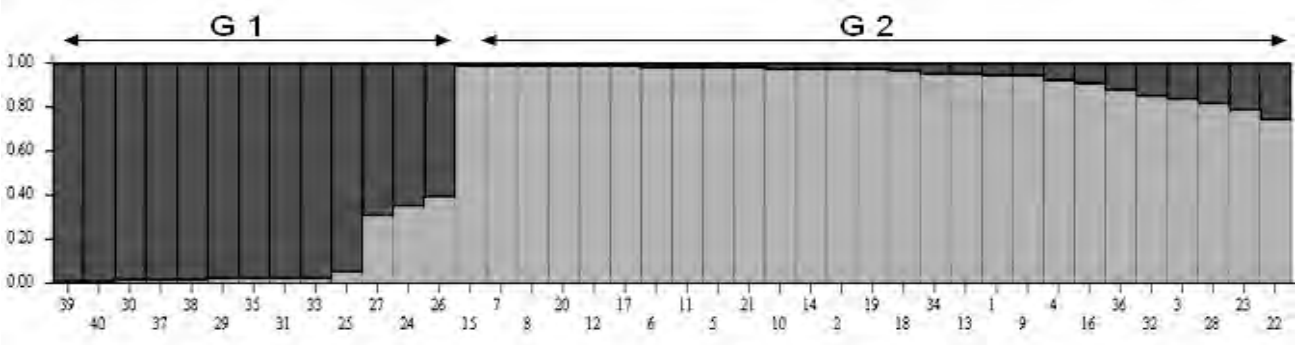

Fig. 2. Population structure analysis of 40 finger millet genotypes generated by STRUCTURE 2.3.2 using the admixture model with correlated allele frequencies. Numbers on the y-axis show the subgroup membership, and the numbers on the x-axis show the genotype number. Different populations are represented by different colors at K=2, where K is the number of subgroups/ subpopulations. Genotype numbers are: 1=GE28, 2= GE29, 3=GE62, 4=GE202, 5=GE595, 6=GE753, 7=GE1736, 8=GE1801, 9=GE174, 10=GE314, 11=GE617, 12=GE1361, 13=GE918, 14=GE580, 15=GE1360, 16=GE68, 17=GE894, 18=GE1924, 19=GE565, 20=GE594, 21=GE468, 22=GE4690, 23=GE4682, 24=GE4725, 25=GE4752, 26=GE4765, 27=GE4796, 28=GE5121, 29= GE5130, 30=GE4915, 31=GE4927, 32=GE4951, 33=GE5025, 34=GE5027, 35=GE5029, 36=GE4990, 37=GE4991, 38=GE4813, 39=GE4833, 40=GE5013

G2) and were classified as members of that sub-group (Table 1), whereas six genotypes (15.0%) were categorized as admixture forms with varying levels of membership shared between the two sub-groups. The mixture is likely the result of breeding (targeted or accidental intercrossing) and domestication history (Africa and India being the primary and secondary center of diversity for finger millet respectively), which have had large effects on the diversity structure. The independent population histories of the groups have shaped gene pools (Garris *et al*., 2005). Sub-groups G1 included 13 (32.5%) genotypes (African genotypes), one each from Tanzania and Ethiopia, two each from Malawi, Zambia and Zimbabwe, and five from Kenya. Sub-group G1 included genotypes from all the African countries except Uganda which were grouped with Indian genotypes. This may be due to the presence of distinct morphological traits in Ugandan genotypes as is evident from our phenotypic data and the distinctness of Ugandan lines was also reported by earlier studies (Arya *et al*., 2013). Whereas, sub-group G2 (dominant in Indian genotypes) included the remaining 27 (67.5%) genotypes, with 21 from India (one each from Karnataka, Kerala and Bihar, two from Madhya Pradesh, three from Uttaranchal, five from Andhra Pradesh and eight from Tamil Nadu), six from Africa (admixtures) [one each from Kenya (GE 5121), Zambia (GE 4951), Zimbabwe (GE 5027) and Tanzania (GE 4990) and two from Uganda (GE 4690, GE 4682)]. Same six accessions were reported as admixtures based on SSR based population structure analysis (Arya *et al*., 2013). This structuring is correlated well with primary and secondary centers of finger millet diversity and the two sub-populations (G1 and G2) basically form two distinct groups and can be used to augment finger millet in both India and Africa.

RAPD marker analysis very well revealed the geographic distribution pattern of genetic variation and population structure in the Indian and African finger millet and also supported the previous reports based on other marker systems emphasizing the role of RAPDs as effective markers for evaluating genetic diversity in finger millet. Our results indicate that Tamil Nadu followed by Andhra Pradesh region of India and in Africa, Zambia followed by Kenya are the diversity rich regions of finger millet. More number of finger millet germplasm should be collected from these places to capture the existing genetic diversity. On the other hand, in other finger millet growing regions, the germplasm may be collected from wider areas to collect existing variability. In summary, the results derived from genetic diversity and population structure analyses could be used for making efficient strategies for finger millet germplasm collection, conservation and designing effective breeding programs to broaden its genetic base.

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Genetic Diversity Studies and Screening for Fusarium Wilt (*Fusarium udum* Butler) Resistance in Wild Pigeonpea Accessions, *Cajanus scarabaeoides* (L.) Thouars

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Wild relatives play an important role in the genetic improvement of cultivated crops. *Cajanus scarabaeoides* (L.) Thouars is one of the important wild species, which possess several desirable traits. Investigations on 67 accessions of *C. scarabaeoides* and 3 cultivated varieties for total soluble seed protein profiles using Sodium Dodecyl Sulphate-Poly Acryl amide Gel Electrophoresis resolved the protein bands ranged from 4-11. Based on the presence or absence of bands, similarity values were calculated and dendrogram was constructed using Unweighted Pair Group Method with Arithmetic Mean analysis. The dendrogram formed two main clusters. The first main cluster consisted of three cultivated varieties and 55 accessions of *C. scarabaeoides* and the second main cluster comprised of remaining 12 accessions of *C. scarabaeoides.* Similarity percentage ranged from 38.5 to 100. ICP15728 is the lone genotype which is distinctly different from remaining genotypes formed separate sub-cluster in the second main cluster with 38.5% similarity. Thirty eight accessions of *C. scarabaeoides* were screened against Fusarium wilt under glass house conditions. Of the 38 accessions screened seven accessions (ICP12707, ICP15689, ICP15692, ICP15732, ICP15744, ICP15748 and ICP15754) showed resistance to Fusarium wilt. Therefore, these accessions could be utilized in breeding programmes to increase the levels of resistance to Fusarium wilt in the pigeonpea cultivars, after further testing.

Key Words: *Cajanus scarabaeoides* accessions, Fusarium wilt, Genetic variability, Resistance screening, Wild species

Introduction

Pigeonpea is an important grain legume crop grown in the tropics and subtropics. The Indian subcontinent, eastern Africa and central America are the world's three main pigeonpea producing regions (Mallikarjuna *et al*., 2011) with Indian subcontinent contributing 75-80% to world's production (Sharma *et al*., 2012).

Pigeonpea is attacked by a range of biotic and abiotic factors, which are major constraints to increase productivity of pigeonpea (Mallikarjuna *et al*., 2011). Among the biotic factors, more than 60 pathogens including fungi, bacteria, viruses, mycoplasma, and nematodes can infect pigeonpea. Of these, Fusarium wilt and Phytophthora blight are widespread across regions (Reddy *et al*., 2012).

Fusarium wilt caused by soil-borne fungus *Fusarium udum* Butler is an important fungal disease prevalent in the pigeonpea growing areas it is more severe in Indian subcontinent (Singh *et al*., 2013). In Indian subcontinent the crop losses ranged from 16 - 47% (Prasad *et al*., 2003) and wilt incidence is believed to have increased significantly over the time (Gwata *et al*., 2006). The annual losses due to wilt have been estimated at US$71 million in India and US$5 million in eastern Africa (Reddy *et al*., 2012).

Although it has been suggested that wilt incidence can be reduced by various practices, host plant resistance would be the most effective, economical and environmental friendly management practice for disease control. There are only few sources of resistance reported for Fusarium wilt (Prasanthi *et al*., 2009; Karimi *et al*., 2010). But the wild relatives of crops are important sources of resistance to biotic and abiotic constraints (Mallikarjuna *et al*., 2007). Pigeonpea offers a rich source of variability in the form of wild relatives, which could be utilized for disease resistance, good agronomic traits, enhancing nutritional quality, identification and diversification of cytoplasmic base of cytoplasmic male sterility (CMS) system etc. (Singh *et al*., 2013).

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Cajanus scarabaeoides, C. acutifolious, C. sericeus and *C. albicans* are some of the wild *Cajanus* species with resistance to pigeonpea pod borer *Helicoverpa armigera* (Sujana *et al.*, 2008). Hence, assessment of genetic variability present in accessions of *C. scarabaeoides* is important for better utilization of germplasm. Successful attempts were made by researchers to study the genetic diversity present in cultivated and wild species which includes seed protein analysis (Ladizinsky and Hamel, 1980; Jha and Ohri, 1996), which are accession specific help in detecting diversity among the wild and cultivated species.

The protein profiling of germplasm and genetic markers have been widely and effectively used to determine the taxonomic and evolutionary aspects of several crops (Murphy *et al*., 1990; Khan, 1990; Das and Mukarjee, 1995; Ghafoor *et al*., 2002). Knowledge of genetic variability present in crop plants is important to plan the breeding programmes. Therefore, this study was designed to assess the genetic variability and also to screen the accessions of *C. scarabaeoides* to identify the Fusarium wilt resistance which could be utilized in pigeonpea disease resistance breeding programmes.

Materials and Methods

Plant Material

The experimental material for genetic diversity study comprised of seeds of 67 accessions of *C. scarabaeoides* collected from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana (Table 1) and three cultivated varieties of pigeonpea *viz.,* Pusa 33, Pusa 855 and Pusa 991. Thirty eight accessions of *C. scarabaeoides*, three cultivated varieties (Pusa 33, Pusa 855 and Pusa 991) and one susceptible variety-Bahar (as a check) was used to screen against Fusarium wilt resistance (Table 2).

Preparation of Protein Samples

The total soluble proteins were analyzed following the procedure described by Laemmli (1970). Five seeds of each genotype were ground to powder and defatted using defatting solvent mixture. Defatting was performed for 9 h with at least three solvent changes and later the ground material was air dried by leaving it open at room temperature overnight. Protein extraction was carried out by homogenizing finely ground cotyledon meal in 25mM Tris-glycine buffer (pH 8.3) overnight. Clear supernatant was decanted after centrifuging it for

Table 1. Details of *C. scarabaeoides* accessions used for total soluble proteins studies

ICP No.	Alternative accession identifier	Origin
12707		India
15683	ICPW 082	India
15684	ICPW 083	India
15685	ICPW 084	India
15687	ICPW 086	India
15688	ICPW 087	India
15689	ICPW 088	India
15690	ICPW 089	India
15691	ICPW 090	India
15692	ICPW 091	India
15693	ICPW 091	India
15694	ICPW 093	Sri Lanka
15695	ICPW 094	Sri Lanka
15696	ICPW 095	Myanmar
15697	ICPW 096	India
15699	ICPW 098	India
15700	ICPW 099	India
15702	ICPW 101;JM 4880	India
15703	ICPW 101;JM 4893	India
15704	ICPW 103;JM 4989	India
15705	ICPW 104;JM 5012	India
15706	ICPW 105;JM 5040	India
15707	ICPW 106; JM 5054	India
15708	ICPW 107; JM 5056	India
15709	ICPW 108;JM 5076	India
15710	ICPW 109	India
15711	ICPW 110	India
15712	ICPW 111	India
15713	ICPW 112	India
15716	ICPW 115	India
15717	ICPW 116	India
15718	ICPW 117	India
15721	ICPW 120	Philippines
15722	ICPW121	India
15724	ICPW 123;RPSP 458	India
15725	ICPW 124;RPSP 827	
15726	ICPW 125	India
15727	ICPW 126	India
15728	ICPW 127	India
15730	ICPW 129	India
15731	ICPW 130; Jangalapalli	India
15732	ICPW 131	India
15733	ICPW 132; ARKS 12347	India
15734	ICPW 133; EC 121206;NT2510	Australia
15735	ICPW 134;EC 121207;NT 2495	Australia
15736	ICPW 135; EC 122342	Fiji
15737	ICPW 136;EC 122344	Fiji
15738	ICPW 137; IW 3339	India
15739	ICPW138;IW 4353	India
15740	ICPW 139;IW 3463	India
15742	ICPW141;IW 2458.	Australia
15744	ICPW143;IW 2496	Australia
15745	ICPW 144; NT2510	Australia
15746	ICPW 145	India
15747	ICPW 146	India
15748	ICPW 147	India
15749	ICPW 148	India
15750	ICPW 149	India
15751	ICPW 150	India
15752	ICPW 151	India
15753	ICPW 152	India
15754	ICPW 153	India
15755	ICPW 154	India
15756	ICPW 155	India
15757	ICPW 156	India
15758	ICPW 157	India
15759	ICPW 158	India

10 min. at 10,000 rpm and 0.2 ml of the Tris-glycine extract was taken and diluted with equal amount of the working sample buffer in each genotype. The mixture was boiled for 10 min., cooled and used for electrophoresis fractionation.

Gel Electrophoresis

The gel of 1mm thickness was prepared by using resolving gel and stacking gel. While preparing the gel all the reagents of resolving gel were mixed well and poured between the plates of the cassette. Care was taken to avoid air bubbles to be trapped in the gel solution and $3/4^{th}$ of the cassette was filled with resolving gel and it was allowed to polymerize. After the resolving gel gets polymerized the reagents of the stacking gel were mixed thoroughly and poured carefully above it and a comb was inserted immediately without trapping any bubble and gel was allowed to polymerize.

Once the gel was polymerized 5µl of the protein sample was loaded in each well using a syringe. The cassette was fitted into the electrophoresis tank and also the lower and upper tanks were filled with electrode buffer. The electrophoresis apparatus was connected to the power pack by fitting the electrodes into sockets of the identical colour. Voltage and power were turned to maximum. Current was adjusted to 1.5 m Amp per well till the tracking dye reached the bottom of the gel.

Gel Fixing and Staining

The gel was carefully removed from the plates after the run and immersed in 150ml of fixing solution (*i.e.* 15% Tri-Chloro Acetic acid-TCA%) overnight. The gel was then rinsed with distilled water and mixture of 15ml of 2% comassie blue and 100ml of 15% TCA was added inside the tray containing the gel. Staining was done till the bands developed well. Later the stain was tipped off and the gel was destained in distilled water till the background was clear. Later the gels were washed and photographed.

Evaluation and Documentation

The electrophorograms were prepared measuring the distance of each band from the point of loading. The relative mobility (Rm) of each band was calculated as ratio of distance traveled by the band to the distance traveled by tracking dye and the bands are numbered on the basis of increasing Rm values.

Calculation of Similarity Indices

Similarity index values were calculated based on proportion of common fragments between two lanes by using the following formula:

$$F = \frac{2Mxy}{Mx+My}$$

Where, F is the similarity index, Mx is the number of bands in accession x, My is the number of bands in accession y and Mxy is the number of bands common to both x and y. Fx100 gives the percent similarity between two accessions, thus F=1.0 would mean that the patterns in the two accessions are identical. Phylogenetic tree indicating the relationship among the genotypes was obtained based on simple matching coefficients using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Numerical Taxonomy System (NTSYS-pc) analysis (Rohalf 1993).

Fusarium udum Spores

A virulent isolate of *F. udum* (spore concentration 10^6spores/g of soil) was collected from Division of Plant Pathology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi.

Raising of Seedlings for Fusarium wilt Resistance Screening

Seeds of *C. scarabaeoides*, cultivated varieties and susceptible variety of pigeonpea were grown in plastic seedling trays containing mixture of autoclaved riverbed sand, virulent isolate of *F. udum* and antibiotic. Seedlings were raised by watering with sterilized water.

Observations for Wilt Incidence

Observations were recorded regularly 15 Days after inoculation (DAI) to 45 DAI. The Percentage wilt incidence (PWI) was calculated as ratio of number of infected to the total number of plants and the genotypes were categorized using a disease rating scale of 1-9 (Nene *et al.*, 1981) as 1: resistant (no symptoms/ 0% incidence); 3: moderately resistant (10% or less incidence); 5: tolerant (11-20% incidence); 7: moderately susceptible (21-50% incidence); and 9: susceptible (51% or more incidence).

Results and Discussion

Genetic Diversity Studies

As a step towards biochemical characterization, 67 accessions of *C. scarabaeoides* along with three cultivated varieties were analyzed for total seed proteins using Sodium Dodecyl Sulphate-Poly Acryl amide Gel Electrophoresis (SDS-PAGE). The electrophoretic profile of total soluble proteins revealed characteristic banding pattern for all the 67 accessions and three cultivated

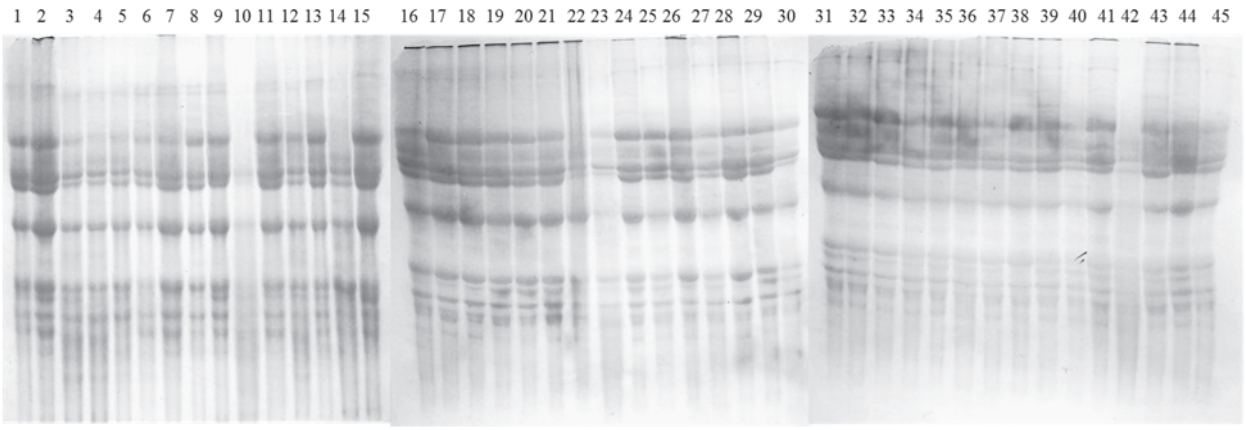

Fig. 1a. Electrophoregrams of accessions of *C. scarabaeoides* for total soluble proteins

Lanes 1-15; ICP15683, ICP15685, ICP15689, ICP15690, ICP15691, ICP15692, ICP15693, ICP15695, ICP15697, ICP15702, ICP15703, ICP15705, ICP15706, ICP15710, ICP15711. Lanes 16-30; ICP15737, ICP15740, ICP15742, ICP15744, ICP15747, ICP15748, ICP15749, ICP15750, ICP15751, ICP15752, ICP15753, ICP15754, ICP15755, ICP15756, ICP15757. Lanes 31-45; ICP15758, ICP15759, ICP15684, ICP15687, ICP15688, ICP15694, ICP15696, ICP15699, ICP15700, ICP15704, ICP15709, ICP15722, ICP15724, ICP15739, ICP15745

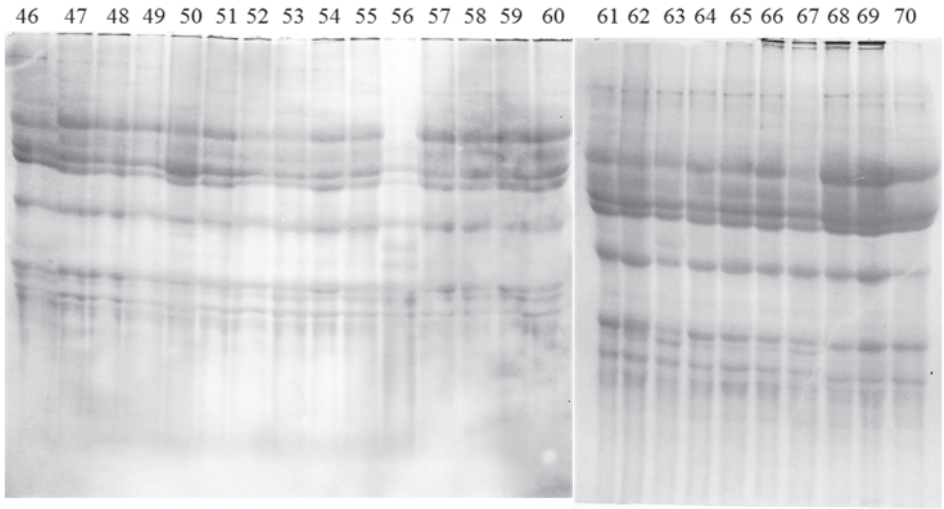

Fig. 1b. Electrophoregrams of accessions of *C. scarabaeoides* and cultivated varieties for total soluble proteins

Lanes 46-70; ICP12707, ICP15712, ICP15713, ICP15716, ICP15717, ICP15718, ICP15721, ICP15725, ICP15726, ICP15727, ICP15728, ICP15731, ICP15732, ICP15733, ICP15735, ICP15746, ICP15707, ICP15708, ICP15730, ICP15734, ICP15736, ICP15738, Pusa-33, Pusa-855, Pusa-991

varieties (Fig. 1a & 1b). The number of bands resolved ranged from 4, in case of ICP15710 to 11 (ICP15747, ICP15748, ICP15751, ICP15752, ICP15758, ICP15699, ICP15700, ICP15724, ICP15739). The band number *viz*., 4 (Rm0.34), 6 (Rm0.46), and 9 (Rm0.62) were common to all the accessions/ genotypes.

Based on the presence or absence of bands, similarity values were calculated for all pair wise comparisons made among 67 accessions of *C. scarabaeoides* and three cultivated varieties using Nei and Lis (1979) equation. The similarity values in percentage ranged from 38.5 to 100. Based on similarity matching coefficients, a dendrogram (phylogenetic tree) was constructed using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) analysis (Fig. 2). The dendrogram formed two main clusters for 67 accessions of *C. scarabaeoides* and three cultivated varieties. The first main cluster consisted of three cultivated varieties

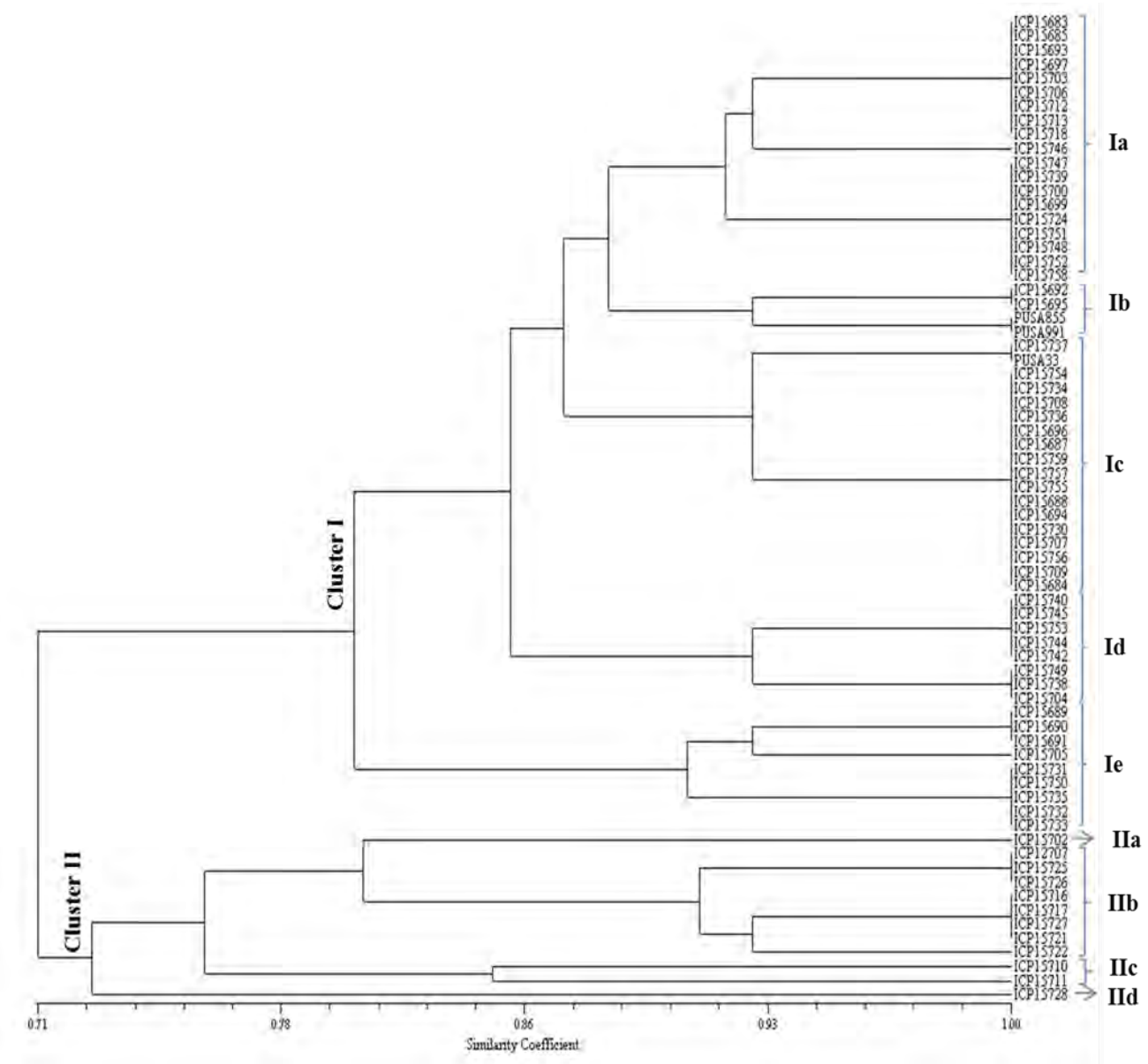

Fig. 2. Dendrogram based on total soluble proteins showing relationship of 67 accessions of *C. scarabaeoides* and three cultivated varieties of pigeonpea

and 55 accessions of *C. scarabaeoides* and the second main cluster comprised of remaining 12 accessions of *C. scarabaeoides.*

The first main cluster was further divided into five sub clusters viz., Ia (consisted of 19 accessions: ICP15683, ICP15685, ICP15693, ICP15697, ICP15703, ICP15706, ICP15712, ICP15713, ICP15718, ICP15746, ICP15747, ICP15739, ICP15700, ICP15699, ICP15724, ICP15751, ICP15748, ICP15752, ICP15758), Ib (two accessions and two cultivated varieties: ICP15692, ICP15695, Pusa-855, Pusa-991), IC (17 accessions & one cultivated variety: ICP15737, ICP15754, ICP15734, ICP15708, ICP15736, ICP15696, ICP15687, ICP15759, ICP15757, ICP15755, ICP15688, ICP15694, ICP15730, ICP15707, ICP15756, ICP15709, ICP15684, Pusa-33), Id (eight accessions: ICP15740, ICP15745, ICP15753, ICP15744, ICP15742, ICP15749, ICP15738, ICP15704), Ie (nine accessions: ICP15689, ICP15690, ICP15691, ICP15705, ICP15731, ICP15750, ICP15735, ICP15732, ICP15733). The second main cluster was also divided in to four sub-clusters *viz.,* sub cluster IIa comprised of only one accession *i.e* ICP15702, sub cluster IIb

consisted of eight accessions *viz*., ICP12707, ICP15725, ICP15726, ICP15716, ICP15717, ICP15727, ICP15721, ICP15722, sub-cluster IIc consisted of two accessions *viz*., ICP15710, ICP15711 and sub cluster IId consisted of only one accession i.e ICP15728 which is diverse from all other genotypes.

Fusarium wilt Resistance Screening

C. scarabaeoides, a wild species of Indian origin, that has many desirable characters (Upadhyaya 2006), has multiple disease resistance (Kulkarni *et al*., 2003; Upadhyaya, 2006), is cross compatible with cultivated pigeonpea and inter-specific gene transfer is possible through conventional hybridization (Mallikarjuna *et al*., 2007). Therefore, in the present study 38 accessions of *C. scarabaeoides* were screened for Fusarium wilt resistance. The percent disease incidence ranged from 0-100% among the accessions of *C. scarabaeoides* (Table 2). Out of 38 accessions screened, seven accessions *viz*., ICP12707, ICP15689, ICP15692, ICP15732, ICP15744, ICP15748 and ICP15754 exhibited resistance with 0% disease incidence, and rest of the accessions showed moderately susceptible to susceptible reaction with percent disease incidence ranging from 22.22 to 100%. Three cultivated varieties (Pusa 33, Pusa 855 and Pusa 991) produced moderately susceptible to susceptible reaction with disease incidence ranging from 44.44 to 77.77% and the susceptible check (Bahar) showed 100% wilt incidence.

Out of seven accessions which exhibited resistant reaction with 0% of disease incidence, six accessions were placed in the first main cluster of the dendrogram (accession ICP15748 was placed at sub-cluster Ia, accession ICP15692 at sub-cluster Ib, accession ICP15754 at sub-cluster Ic, ICP15744 at sub-cluster Id and ICP15689, ICP15732 at sub-cluster Ie) and one accession ICP12707 at the sub-cluster IIb of the second main cluster. ICP15692 accession displayed resistance to Fusarium wilt in this study was also found to be resistant to both the mild and severe strains of *Pigeonpea sterility mosaic virus* in the earlier studies of Kulakarni *et al*. (2003).

A considerable amount of variation was observed based on SDS-PAGE that indicated the utilization of seed protein markers for germplasm classification in pigeonpea. SDS-PAGE of total soluble seed proteins was able to characterize and identify all the studied genotypes and can be employed effectively for identification of pigeonpea genotypes. This technique was employed by Drzewiecki (1990) in pea, Mudzana *et al*. (1995) in faba bean, Goyal and Sharma (2003) in cluster bean and these scientists successfully showed protein electrophoresis as powerful tool to identify the crop plants. For any successful crop improvement programmes characterization of variability present in wild relatives/ species is important. To assess the genetic variability present in crops and their wild relatives/ species total soluble proteins as biochemical markers can be employed.

Many studies were done previously on screening for resistance to Fusarium wilt and other diseases to identify the resistance source. To mention a few, Nene and Kannaiyan (1982) conducted experiment on field screening of more than 11,000 entries of pigeonpea and found 33 lines to be resistant to *F. udum*. Saxena *et al*. (1990) evaluated 33 accessions of *Atylosia scarabaeoides* at ICRISAT and detected accessions resistant to Fusarium wilt, Phytophthora blight, sterility mosaic and cyst nematode (*Heterodera* sp.). Prasanthi *et al*. (2009) screened 88 lines along with ICPL87119 and ICPL8863 resistant checks for Fusarium wilt under field conditions and artificial inoculation and identified 14 lines having 0-20% plant mortality. Jaagrati Jain (2006) tested 35 genotypes for multiple disease and insect resistance and identified five genotypes with multiple disease resistance to Fusarium wilt and sterility mosaic. Kumar *et al*. (2005) evaluated 115 wild *Cajanus* accessions belonging to six species viz. *C. albicans, C. platycarpus, C. cajanifolius, C. lineatus, C. scarabaeoides* and *C. sericeus* under greenhouse conditions in endemic locations of each isolate through mite-mediated virus inoculation against three *Pigeonpea sterility mosaic virus* isolates prevailing in peninsular India and found 15 accessions (ICP 15614, 15615, 15626, 15684, 15688, 15700, 15701, 15725, 15734, 15736, 15737, 15740, 15924, 15925 and 15926) showing resistance to all three isolates.

Seven accessions of *C. scarabaeoides viz.*, ICP12707, ICP15689, ICP15692, ICP15732, ICP15744, ICP15748, and ICP15754 showed resistance to Fusarium wilt caused by *F. udum* in this investigation could be utilized as a source of resistance in breeding programmes to increase the levels of resistance to Fusarium wilt in the pigeonpea cultivars after further testing. This will be of great help to pigeonpea breeders to develop resistance breeding programmes.

Table 2. Percent wilt incidence in the accessions of *C. scarabaeoides* and cultivated varieties of pigeonpea

Genotypes	Alternative accession identifier	Origin	Percent wilt incidence (PWI)
ICP12707		India	0
ICP15683	ICPW 082	India	25
ICP15685	ICPW 084	India	25
ICP15689	ICPW 088	India	0
ICP15691	ICPW 090	India	33.33
ICP15692	ICPW 091	India	0
ICP15693	ICPW 091	India	33.33
ICP15695	ICPW 094	Srilanka	71.42
ICP15697	ICPW 096	India	80
ICP15702	ICPW 101; JM 4880	India	100
ICP15703	ICPW 101; JM 4893	India	100
ICP15706	ICPW 105; JM 5040	India	100
ICP15710	ICPW 109	India	25
ICP15711	ICPW 110	India	100
ICP15712	ICPW 111	India	22.22
ICP15713	ICPW 112	India	100
ICP15716	ICPW 115	India	100
ICP15717	ICPW 116	India	75
ICP15718	ICPW 117	India	66.66
ICP15721	ICPW 120	Philippines	100
ICP15725	ICPW 124; RPSP 827	India	100
ICP15726	ICPW 125	India	100
ICP15728	ICPW 127	India	50
ICP15731	ICPW 130; jangalapalli	India	60
ICP15732	ICPW 131	India	0
ICP15733	ICPW 132; ARKS 12347	India	100
ICP15737	ICPW 136; EC 122344	Fiji	100
ICP15740	ICPW 139; IW 3463	India	100
ICP15742	ICPW141; IW 2458.	Australia	42.85
ICP15744	ICPW143; IW 2496	Australia	0
ICP15747	ICPW 146	India	80
ICP15748	ICPW 147	India	0
ICP15749	ICPW 148	India	33.33
ICP15751	ICPW 150	India	100
ICP15752	ICPW 151	India	100
ICP15753	ICPW 152	India	50
ICP15754	ICPW 153	India	0
ICP15755	ICPW 154	India	100
Pusa 33			50
Pusa 855			44.44
Pusa 991			77.77
Bahar			100

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Assessment of Diversity in Commercial Hybrids of Pearl Millet in India

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Availability of diverse cultivars is essential in order to meet necessity of regional adaptation to various climatic conditions and to fulfil the farmers' need of differential preference of various phenotypic traits in pearl millet. The present study attempted to quantify the degree of diversity in commercial hybrids of pearl millet and to understand the relationship among various phenotypic and quality traits. A total of 122 commercial hybrids were evaluated at three diverse locations. Results showed large variation for flowering time (42-58 days), tillering (1.1-4.4 panicles/plant), individual grain size (7.6-17.3 mg), plant height (185-268 cm), panicle length (20-33 cm) and grain yield (35-72 q/ha). Clustering of hybrids resulted in 7 distinct clusters, which highlighted the successful efforts of national programme of pearl millet improvement toward genetic diversification of hybrids. These results are discussed as to how this reported diversity has helped in extending the hybrid technology in some of the most difficult and diverse production ecologies. Phenology, tillering and height had significant influence on yields of hybrids. Taller hybrids provided higher yield. Hybrids with lesser duration and shorter height produced more tillers and tillering decreased with the increase in panicle length. The correlation of grain yield with Fe and Zn concentration in grains was not significant suggesting that both these micronutrients can be improved without significantly compromising on grain yield.

Key Words: Adaptation, Genetic diversity, Hybrids, Pearl millet, Trait association

Introduction

Pearl millet (*Pennisetum glaucum* L. R. Br.) is the most important dryland cereal in arid and drier semi-arid tropics. It is an essential component of staple food in parts of India and sub-Sahara Africa and its dry stover is significant component of ration to a large number of bovine livestocks especially during dry periods of year. Its nutritious grains contain higher iron (Fe) and zinc (Zn) than rice, wheat and maize (Rai *et al*., 2008). There is also increasing utilization of pearl millet grain in non-food uses like feed and alcohol extraction (Basavaraj *et al*., 2010).

Pearl millet is grown in diverse agro-ecologies ranging from highly drought-prone north-western parts of India to relatively favourable southern regions of country. Availability of diverse cultivars is essential in order to meet necessity of regional adaptation to various climatic stresses and to fulfil the need of differential preference of various phenotypic traits by farmers of different areas (Yadav *et al*., 2012). Genetic diversity is also very vital in disease management, especially downy mildew, which is the most destructive disease of genetically homogeneous hybrids of pearl millet.

Pearl millet hybrid breeding is one of the most successful programmes in India (Gill, 1991; Dave, 1987; Harinarayana *et al*., 1999; Yadav and Rai, 2013). Large number of hybrids, developed by both public and private sector organizations, have been widely adopted by Indian farmers (Yadav *et al*., 2012). However, little information exists on magnitude of diversity in commercial hybrids, though some information is available on extent of genetic diversity in landraces (Vyas and Pokhriyal, 1985; Appa Rao *et al*., 1986), inbreds (Virk, 1988) and experimental crosses (Pethani and Dave, 1992; Khairwal and Singh, 1999). Therefore, current study attempted to quantify the degree of diversity in commercial hybrids of pearl millet. The relationship among various phenotypic traits and between grain yield and quality traits was also examined.

Materials and Methods

The experimental material consisted of 122 hybrids including 21 hybrids from nine public sector research

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organizations and 101 hybrids from 33 seed companies. While the hybrids from public sector were all released and notified, those from the private sector consisted of both released and notified for general cultivation as well as those marketed as truthfully labelled seed. The seed of the hybrids used in this experiment were obtained from respective institutes and organizations that developed those hybrids.

Hybrids were evaluated at the All India Coordinated Pearl Millet Improvement Project (AICPMIP), Jodhpur, Rajasthan; CCS Haryana Agricultural University, Hisar, Haryana; and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana. Each site represented one of the three mega-environments of pearl millet production ecology: A1 zone was represented by Jodhpur, A zone by Hisar and B zone by Patancheru (Gupta *et al.*, 2013). The soils were sandy, loamy and sandy loam at Jodhpur, Hisar and Patancheru, respectively.

At each site, hybrids were evaluated in three replications using alpha-lattice design in 4 m long 4-6 row plots during rainy season of 2012. The hybrids were sown in plots followed by thinning of seedlings within first two weeks of sowing to maintain a final spacing of 15 cm within row that were spaced 60 cm apart at Jodhpur and Hisar and 75 cm at Patancheru. The trials were grown under good management conditions to allow optimal expression of hybrids.

Data were recorded for grain yield, five phenotypic traits and two grain quality traits. Days to flowering were recorded on plot basis as number of days from sowing till time the stigma emerged in the main panicle of 50% plants in a plot. Plant height and main panicle length were recorded at the time of maturity on five competitive plants in a plot. Data on plant stand and panicle number were used to calculate panicles per plant. At the time of maturity, the panicles were harvested and put in gunny bags for drying, rather than on ground, to avoid any contamination with dust. Grain yield was recorded on plot basis at Jodhpur and Patancheru and converted into kg/ha. Part of grain samples from these two locations were used to record 1000-grain weight and for analysis of Fe and Zn concentration using Inductively Coupled Plasma Optical Emission Spectroscopy (Wheal *et al.*, 2011) at the Waite Analytical Services Laboratory, Australia.

Data recorded on all traits were subjected to analysis of variance at individual location and across locations. Association of various traits with grain yield was studied using Pearson's correlation coefficients. Hybrids were grouped in clusters using five highly heritable phenotypic traits viz., days to flower, plant height, panicle length, panicles/plant and 1000-grain weight. Pooled data for each trait was used for calculating pair-wise genetic dissimilarity index following the method of Euclidean distance using Ward's minimum variance method (Ward, 1963).

Results and Discussion

Magnitude of Variation

The climatic conditions at three test locations varied considerably resulting in variable expression of all traits at different locations (Table 1). Diverse growing conditions resulting in differential expression of traits at three locations indicated that the results of this study may be applicable to a varied range of environments under which pearl millet is cultivated in various agro-ecologies of country.

The mean flowering time of hybrids at Patancheru was 45 days, which was 7-13 days lesser than the mean flowering time recorded at Jodhpur and Hisar (Table 1). This was due to shorter day length at Patancheru that resulted in early flowering as pearl millet is a short-day plant (Burton, 1983). This also resulted in shorter plant height (189 cm) at Patancheru than at other two locations. Both tillering and panicle length were also variable across test locations. Plant tillered most profusely at Patancheru followed by Hisar and Jodhpur. However, mean panicle length was maximum at Hisar (26 cm) and minimum at Patancheru (23 cm). The mean grain yield of hybrids ranged from 4880 kg/ha at Jodhpur and 6110 kg/ha at Patancheru.

Hybrids differed significantly for all traits at individual location and across locations (data not presented). Enormous variation was observed for flowering time from 42 to 58 days. Five hybrids

Table 1. Trait means in pearl millet evaluated at three locations during rainy season of 2012

Trait	Jodhpur	Hisar	Patancheru
Time to flower (days)	52.2	57.9	44.9
Plant height (cm)	253.6	242.1	188.6
Panicles/plant (No.)	1.61	1.92	2.13
Panicle length (cm)	24.1	26.2	23.0
Grain yield (kg/ha)	4880	–	6110
1000-grain weight (g)	10.3	–	13.0

– No data

flowered within 45 days, while 30 hybrids (25%) took 46-50 days for flowering (Fig. 1). Half of the hybrids flowered between 51 and 55 days and remaining hybrids (19) could flower beyond 55 days. Such a wide range in flowering time showed that current hybrids could cater to the need of highly drought-prone regions in north-western India requiring early maturing hybrids to escape terminal drought (Bidinger *et al*., 1987) and also of relatively more favourable areas where slightly longer duration helps in capturing additional moisture resources for crop (van Oosterom *et al*., 2003).

Large variability was also observed for tillering (1.1-4.4 panicles/plant) and seed size with 1000-grain weight ranging from 7.6 to 17.3 g. High tillering hybrids get a strong preference by farmers of drought-prone areas in the states of Rajasthan, Gujarat and Haryana as tillering is a part of mechanism of adaptation to intermittent drought periods (van Oosterom *et al.*, 2006; Yadav *et al.*, 2011) that are of common occurrence during the crop season (Yadav *et al*., 2011). Most of the hybrids produced large grains with a 1000-grain weight of >10 g. This is a reflection of a high priority given in pearl millet breeding programme of the country for bold seeds which find favour with vast majority of farmers cultivating pearl millet. There was also a wide range in height (185-268 cm) and panicle length (20-33 cm) among hybrids.

Grain yield of hybrids varied more than two fold from 3.5 to 7.2 t/ha. About 25% of hybrids yielded more than 6.0 t/ha. Available environmental resources like soil type, water availability and input supply determine the crop productivity. The yielding ability of hybrids up to 7.2 t/ha demonstrated the potential productivity of present pearl millet hybrids. The realized grain yield at farmers' field during summer season in parts of Gujarat, Uttar Pradesh and Rajasthan is up to 5.5 t/ha which makes pearl millet a commercial rather than a subsistence crop.

Clustering of hybrids resulted in seven distinct clusters (Fig. 2) with considerable diversity within each cluster. The size of the individual clusters varied considerably. Two largest clusters contained 24-27 hybrids, while three clusters included 17-19 hybrids. Remaining two clusters had 8-9 hybrids. It was interesting to note that 14 of 20 maturing hybrids bred for drought prone environments from public sector clustered in Cluster 1, though the remaining six hybrids were spread in three clusters. The hybrids developed by different private companies were spread across seven different clusters that underlined very diverse nature of the individual hybrids bred by various organizations. Though this study assessed diversity of hybrids on the basis of phenotypic traits, there are reports (Nepolean *et al*., 2012; Gupta *et al*., 2015) of high molecular diversity among the parental lines that have been widely used by both public and private sector in developing most of the hybrids tested in this study.

Spread of 122 hybrids in 7 mega-clusters clearly indicated wide diversity in commercial hybrids of pearl millet highlighting the successful efforts of national programme of pearl millet improvement towards genetic diversification of hybrids. This might be the key reason that during last 25 years, there has been no downy mildew epidemic in India unlike those witnessed frequently before 1990. Otherwise, the downy mildew had become most important biotic constraint during 1970s and 1980s. A critical appraisal of the situation (Dave, 1987; Yadav and Rai, 2013) revealed that downy mildew epidemics in mid 1970s and 1980s were mainly due to lack of diversity in hybrids. During 1965-75, all hybrids were first based on Tift 23A and then on 5141A. Similarly, the same restorers were also repeatedly used in combinations with different male-sterile lines. Three restorers (J 104, K 560 and K 559) were male parents of nine commercial hybrids. Similarly two restorers (H90/4-5 and H77/833-2) were utilized in developing five hybrids. These facts indicate that outbreaks of downy mildew were due to a narrow genetic base among seed parents and restorers (Yadav *et al*., 1993; Yadav, 1996).

Thus investment made in genetic diversification of pearl millet hybrids by Indian programme has paid enormous dividends not only by containing the downy mildew but also by providing a wide range of choice to farmers to select hybrids that fulfil their requirements of local adaptation and other preferred phenotypic traits. This is the major reason of achieving higher growth in productivity of pearl millet as compared to that in wheat, rice, sorghum and maize (Yadav and Rai, 2013) during last 25 years. Use of diverse and downy mildew resistant genetic material in the development of both seed and restorer parents would be a continual necessity to further promote the genetic diversity in hybrid breeding.

The Fe density among the hybrids varied from 31 to 61 mg/kg with mean concentration of 45 m/kg. On the other hand, the Zn density ranged from 32 to 52 mg/kg with mean Zn concentration of hybrids being 41 mg/kg. The wide range in the concentration of both Fe

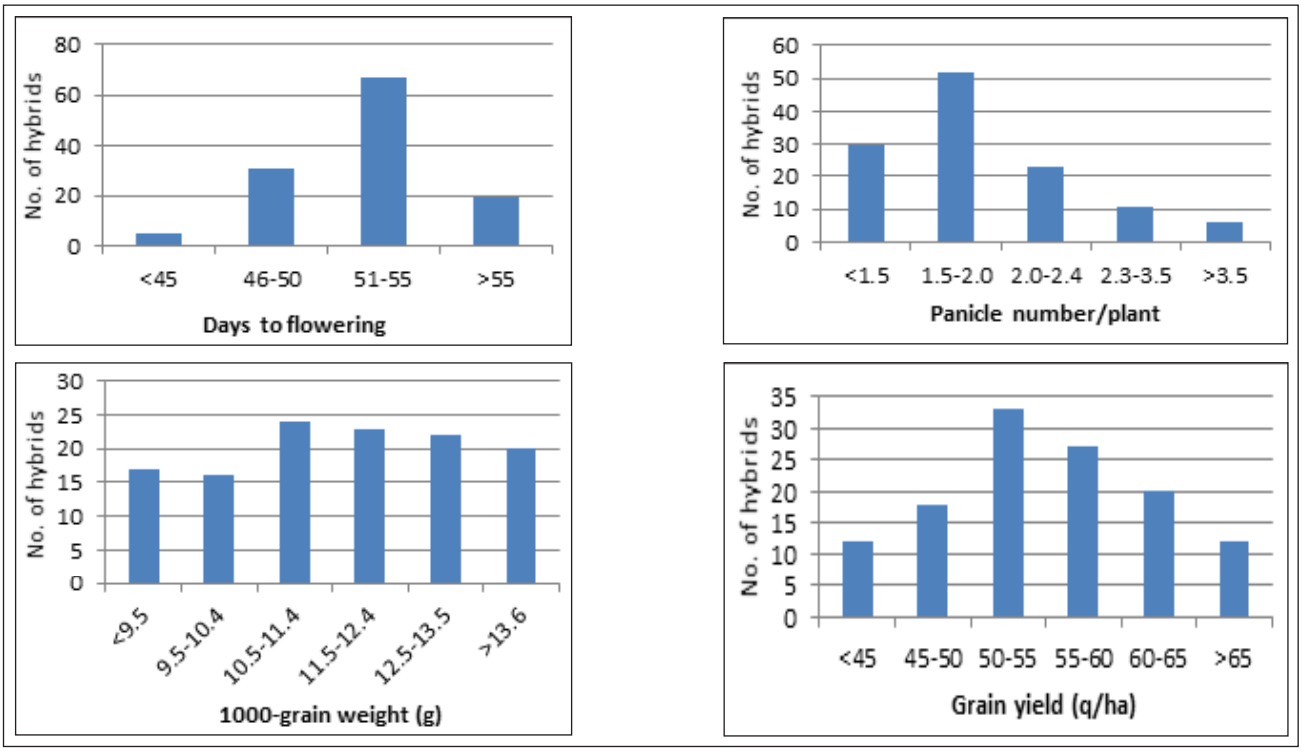

Fig. 1. Frequency distribution of pearl millet hybrids in different classes for grain yield and three phenotypic traits

and Zn among pearl millet hybrids showed that there existed good choice in commercial cultivars for selecting high yielding hybrids coupled with high concentration of these micronutrients.

Association among Traits

Phenology, tillering and plant height had significant influence on grain yield of hybrids. The correlation between grain yield and days to flowering was significant and positive (Table 2), though lateness could explain only 12% of variation in grain yield. This showed that late flowering hybrids tended to yield higher which was expected since moisture was not a limiting factor during evaluations. These results, however, need to be interpreted with slight caution as extending duration beyond a limit (say 85 days) will increase the chances of terminal drought in rainfed crop like pearl millet.

Taller hybrids gave higher grain yield as indicated by highly significant positive association between height and grain yield (Table 2). It is well established in cereals that greater height results in greater biomass that has direct influence on grain yield (Austin *et al.*, 1993). Tillering in hybrids was strongly associated with their duration, tallness and panicle length. Hybrids with lesser duration and shorter height produced more tillers as indicated by significant negative association of panicles per plant with days to flowering and plant height. Tillering decreased with the increase in panicle length as showed by significant negative correlation between panicle length and panicle number per plant. Such compensation in yield components is a part of yield formation process (Austin *et al.*, 1993) and has direct relevance in prioritizing the traits during selection and cultivar development.

The correlation of grain yield with both Fe and Zn concentration was non-significant (Table 3) suggesting that both these micronutrients can be improved without significantly compromising on grain yield. A highly significant and positive correlation was observed between grain Fe and Zn densities of hybrids which is in conformity with earlier results obtained with a wide range of diverse material (Velu *et al.*, 2007, 2008; Rai *et al.*, 2012). Grain size had moderate to weak but positive correlation with Fe and Zn contents and no adverse effect on grain yield. These results clearly indicated that it should be possible to develop large-seeded hybrids with higher Fe and Zn concentration without compromising their yielding ability.

The results of this study have clearly indicated that there is enough diversity in commercial hybrids of pearl millet to cater to the need of diverse agro-ecology of

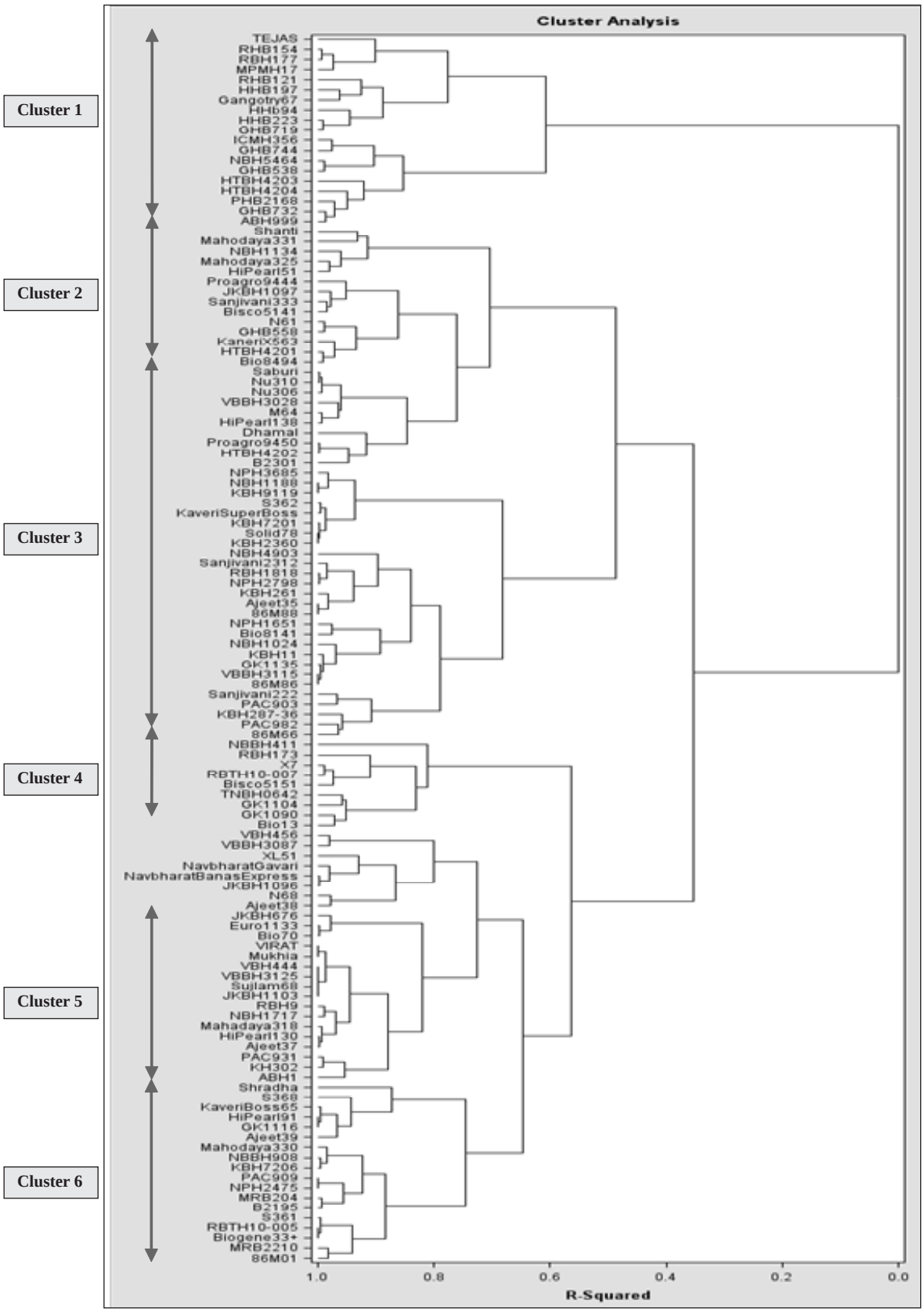

Fig. 2. Clustering of 122 hybrids of pearl millet following the method of Euclidean distance

Table 2. Correlation coefficients among four phenotypic traits and grain yield in 122 hybrids based on mean at three locations

Trait	Days to flowering	Plant height	Panicles/plant	Panicle length	Grain yield
Days to flowering	1.00				
Plant height	0.72**	1.00			
Panicles/plant	-0.57**	-0.46**	1.00		
Panicle length	0.16	0.27*	-0.42**	1.00	
Grain yield	0.36**	0.34**	-0.28*	0.04	1.00

*, ** significant at probability levels of 5% and 1%, respectively

Table 3. Correlation coefficients among grain yield and three seed traits in 122 hybrids based on mean at three locations

Trait	1000 seed weight	Fe concentration	Zn concentration	Grain yield
1000- seed weight	1.00			
Fe content	0.18	1.00		
Zn content	0.31**	0.70**	1.00	
Grain yield	0.12	-0.09	-0.03	1.00

*, ** significant at probability level of 1%

crop. Cultivation of a large number of diverse hybrids has also helped in containing the downy mildew that was threatening hybrid technology *per se* in pearl millet a few decades back. Availability of this diversity would also play a critical role in adaptation to situation emerging out of climate change.

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SSR-Based Genetic Diversity Assessment Among Hexaploid Wheat (*Triticum aestivum* L.) Cultivars

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Genetic diversity of the eighteen wheat (*Triticum aestivum* L.) cultivars were evaluated using 21 SSR markers. A total of 273 alleles were detected and the mean of alleles in each genome was 7.3 (A genome), 8.6 (B genome) and 9.3 (D genome). The polymorphism information content (PIC) value were ranged from 0.630 (*Xgdm132*) to 0.928 (*Xgwm190* and *Xgwm538*) with an average of 0.848. The Pair-wise genetic similarity matrix was calculated based on all possible pairs of wheat cultivars, which varied from 0.56 (VL 914 vs Mons ald's) to 0.97 (Halna vs Iepaca rabe and Kauz/AA/Kauz vs Halna). In UPGMA based cluster analysis using Dice coefficients, 18 cultivars were grouped into 4 major groups and clearly reflected the presence of very high degree of genetic diversity. Therefore, the molecular assessment of the genetic diversity between cultivars should be a good critrion for selecting these cultivars in future breeding programs.

Key Words: Genetic diversity, Microsatellite, Molecular markers, Wheat

Introduction

Wheat (*Triticum aestivum* L.) is one of most important, widely-cultivated cereal crops on a global scale in terms of total harvested weight and amount used as nutrition for human and animal (FAO, 2015). It is one of the main cereal crops in India. It is grown in about 30.60 million ha area with a production of 88.94 million tonnes and productivity of 3.0 tonnes/ha (Anonymous, 2015). Currently, India is second largest producer in the world after China with total 12% share in world wheat production. The world's major wheat producing countries are China, India, USA, France, the Russian Federation, Turkey, UK, Pakistan, Argentina, Canada, Australia, Iran and Italy. These countries together contribute about 76% of the global wheat production (FAO, 2015). In wheat the availability of variability at DNA level is a pre-requisite for any breeding improvement program targeted towards the improvement of its production and productivity. To estimate genetic diversity in wheat germplasm, various kinds of biomolecules based markers can be used. A number of methods are currently used for analysis of genetic diversity in germplasm accessions, recombinant inbreed lines (RILs) and segregating populations. These methods are based on pedigree records, morphological traits, biochemical and molecular markers (Kotzamanidis *et al.,* 2011).

Morphological traits can be used to evaluate genetic diversity but are often influenced by environmental factors and are unreliable, while the expression of molecular markers is the direct product of genes. In wheat, many breeding programs all over the world are aimed to improve for high grain yield with better quality, disease-resistance and agricultural performance with the use of molecular markers. Large number of germplasm lines can be characterized in a short period of time with more accurate assessment of genetic variability than the any other methods using DNA based markers. DNA based markers are more useful due to highly abundant, widely distributed, hypervariability, multiallelic and co-dominant in nature which facilitates the study of genetic divergence (Gorji and Zolnoori, 2011; Valentina *et al.*, 2012), gene mapping (Feuillet and Eversole, 2007) and testing of authenticity of genetic stocks in wheat (Pestsova *et al.,* 2000).

Simple sequence repeats (SSRs) or microsatellites are short tandemly repeated sequences of 2-6 deoxy-nucleotides (Mclauchlan *et al.,* 2001). These are ubiquitous in eukaryotic genomes and the loci are highly polymorphic than the other markers due to the change in the number of repeating units among the individuals of population and each microsatellite locus can easily be amplified using polymerase chain reaction knowing the

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DNA sequence flanking the specifically tandem repeat region. Limitation in the application of SSR markers is the requirement of prior sequence information for the development of primers for locus-specific amplification (Dourar and Akkaya, 2001). The microsatellite markers are extremely useful for many different applications in wheat breeding programmes due to their high level of polymorphism and easy handling (Zeb *et al.,* 2009; Sehgal *et al.,* 2012). Therefore, present investigation was conducted to assess genetic diversity using SSR markers in 18 different wheat cultivars. Thus, the information generated could be useful to improve genetic diversity assessment and their use in plant breeding programs (Bertini *et al.,* 2006).

Materials and Methods

Plant Materials and DNA Extraction

Eighteen wheat cultivars used for the evaluation of genetic diversity were chosen to represent material from diverse sources (Table 1). Leaves from 7-10 days old wheat seedlings were used for genomic DNA extraction. DNA was isolated using CTAB method (Doyle and Doyle, 1987). The quantity of DNA was measured with a UV spectrophotometer at 260 nm and adjusted to a concentration of 50 ng/µl and stored in -20 ^{o}C.

SSR Analyses

The 21 SSR (*Xgwm, Xgdm, Xpsp, Barc* and *Wmc*) markers specific for wheat chromosomes were used for diversity analysis (Table 2). The details of SSR primers were gathered from Grain genes: a database for *Triticeae* and *Avene* (http://graingenes.org) and PCR procedure was used as described by Roder *et al.* (1998). The polymerase chain reaction (PCR) was performed in a volume of 15 µl, containing, 0.5U of Taq DNA polymerase, 1.7 mM dNTPs, 1.5 mM $MgCl_2$, 1X PCR buffer, 3 µM of forward and reverse primer, 50 ng of template DNA and remaining nuclease free water. The amplification was carried out in thermocycler from Biometra, Germany with initial denaturation at 94°C for 7 min, denaturation at 94°C for 30 sec, annealing at 51-61°C for 30 sec, extension at 72°C for 30 sec and final extension at 72°C for 10 min followed by 36 cycles. After PCR amplification, the 15 µl samples were loaded in amount of with 2 µl of 6X loading dye (0.03% bromophenol blue and 0.03% xylene cyanol FF) in 1.5% agarose gel and run at 95 V and 400 mA for about one hour. A DNA standard (Fermentas, Lithuania) marker of 50 bp loaded along with samples for determination of amplification product sizes and documented using gel documentation system from Bio-Rad (Berkeley, U.S.A.).

Data Collection and Diversity Analysis

Marker Polymorphism

The informativeness of the SSR markers was determined using polymorphism information content (PIC). The PIC content was calculated as described by Anderson *et al.* (1993).

Table 1. Wheat cultivars and their sources used in present investigation

Cultivars	Source	Pedigree	Country of origin
Mons Ald's	IARI, New Delhi	-	-
HD 2285	IARI, Pusa	249/HD2150 //HD 2186	India
Sonalika	IARI, New Delhi	II54-388 /AN/3/YT54/N10B/LR 64	India
Iepaca rabe	IARI, New Delhi	-	-
AKAW 4008	IARI, New Delhi	-	-
Halna	IARI, New Delhi	HD 1982/K816	India
Pusa gold	RAU, Pusa	KALYANSONA/HD 1999//HD 2204/DW 38	India
HD 2733	IARI, Pusa	ATTILA /3/ TUI /CARC // CHEN / CHTO /4/ ATTILA	India
PBW 343	RAU, Pusa	ND/VG9144//KAL/BB/3/YCO"S' /4/VEE#S "S"	India
C 306	RAU, Pusa	RGN/CSK3//2*C591/3/C217/N14 //C281	India
Kauz/AA/Kauz	IARI, New Delhi	JUP/BJY//URES	Mexico
Raj 3765	RAU, Pusa	HD 2402/VL639	India
HD 2888	RAU, Pusa	C 306/*T.sphaerococcum*//HW 2004	India
VL 914	IARI, New Delhi	-	India
F5-995	NBPGR, New Delhi	-	-
K0583	RAU, Pusa	-	India
SAWSN 3010	NBPGR, New Delhi	-	-
Cuo/79/Prulla	IARI, New Delhi	-	-

Table 2. SSR locus, their sequences, chromosomal location (Cl), repeat motif and annealing temperature (AT)

Locus	Sequence (5′-3′)	Cl	Repeat motif	AT (^{o}c)
Xgwm44	(L) GTTGAGCTTTTCAGTTCGGC (R) ACTGGCATCCACTGAGCTG	7D	$(GA)_{28}$	60
Xgwm160	(L)TTCAATTCAGTCTTGGCTTGG (R) CTGCAGGAAAAAAAGTACACCC	4A	$(GA)_{21}$	60
Xgwm190	(L) GTGCTTGCTGAGCTATGAGTC (R) GTGCCACGTGGTACCTTTG	5D	$(CT)_{22}$	60
Xgwm261	(L) CTCCCTGTACGCCTAAGGC (R) CTCGCGCTACTAGCCATTG	2D	$(CT)_{21}$	55
Xgwm265	(L) TGTTGCGGATGGTCACTATT (R) GAGTACACATTTGGCCTCTGC	2A	$(GT)_{23}$	55
Xgwm272	(L) TGCTCTTTGGCGAATATATGG (R) GTTCAAAACAAATTAAAAGGCCC	5D	$(CA)_{17}$	50
Xgwm408	(L) TCGATTTATTTGGGCCACTG (R) GTATAATTCGTTCACAGCACGC	5B	$(CA)_{22}(TA)(CA)_{7}(TA)_{9}$	55
Xgwm413	(L) TGCTTGTCTAGATTGCTTGGG (R) GATCGTCTCGTCCTTGGCA	1B	$(GA)_{18}$	60
Xgwm437	(L) GATCAAGACTTTTGTATCTCTC (R) GATGTCCAACAGTTAGCTTA	7D	$(CT)_{24}$	50
Xgwm 499	(L) ACTTGTATGCTCCATTGATTGG (R) GGGGAGTGGAAACTGCATAA	5B	$(GA)_{32}$	60
Xgwm513	(L) ATCCGTAGCACCTACTGGTCA (R) GGTCTGTTCATGCCACATTG	4B	$(CA)_{12}$	60
Xgwm538	(L) GCATTTCGGGTGAACCC (R) GTTGCATGTATACGTTAAGCGG	4B	$(GT)_{6}(T)(GT)_{10}$	60
Xgwm570	(L) TCGCCTTTTACAGTCGGC (R) ATGGGTAGCTGAGAGCCAAA	6A	$(CT)_{14}(GT)_{18}$	60
Barc55	(L) GCGGTCAACACACTCCACTCCTCTCTC (R) CGCTGCTCCCATTGCTCGCCGTTA	1B, 2B, 5B	$(ATCT)_{10}$	55
Barc75	(L) AGGGTTACAGTTTGCTCTTTTAC (R) CCCGACGACCTATCTATACTTCTCTA	3B	$(TAG)_{2}(TAGA)_{5}$	52
Wmc341	(L) ACATGGGCTACATGAGAGAAGA (R) AGAGTGGCTCCCTTTTCACCTA	6B	$(CA)_{24\ 55\ to\ 102}$	61
Wmc397	(L) AGTCGTGCACCTCCATTTTG (R) CATTGGACATCGGAGACCTG	6B	$(CA)_{9\ 69\ to\ 86,}\ (GA)_{14\ 93\ to\ 120}$	61
Xbarc71	(L) GCGCTTGTTCCTCACCTGCTCATA (R) GCGTATATTCTCTCGTCTTCTTGTTGGTT	3D	$(TAGA)_{7}(TA)_{2}$	55
Xbarc198	(L) CGCTGAAAAGAAGTGCCGCATTATGA (R) CGCTGCCTTTTCTGGATTGCTTGTCA	6B	$(ATT)_{19}$	50
Xgdm132	(L) ACCGCTCGGAGAAAATCC (R) AGGGGGGCAGAGGTAGG	6D	$(CT)_{24}$	60
Xpsp3071	(L) CGTGCCCTACACCTCCTTTTCTCTC (R) TCCGTACATACTCCGGGAGACC	6A	$(TC)_{14}$	61

$$PIC = 1-\sum_{j=1}^{k} P^{2}_{ij}$$

Where k is the total number of alleles detected for a given marker locus and P_{ij} is the frequency of the j^{th} allele for i^{th} marker in the set of eighteen wheat cultivars.

Genetic Similarity Estimation and Cluster Analysis

The amplified DNA bands were scored as 1 for presence and 0 for absence for each SSR marker (Fig.1) and entered into binary matrix as discrete variables. The average SSR difference between cultivars were calculated using Dice genetic similarity (GS) coefficient (Dice, 1945) using the NTSYS-pc software ver. 2.1 from Exeter Software, East Setauket, NY. The GS between two cultivars i and j is equivalent to the formula:

$$GS\ [Dice] = \frac{2a}{(2a + b + c)}$$

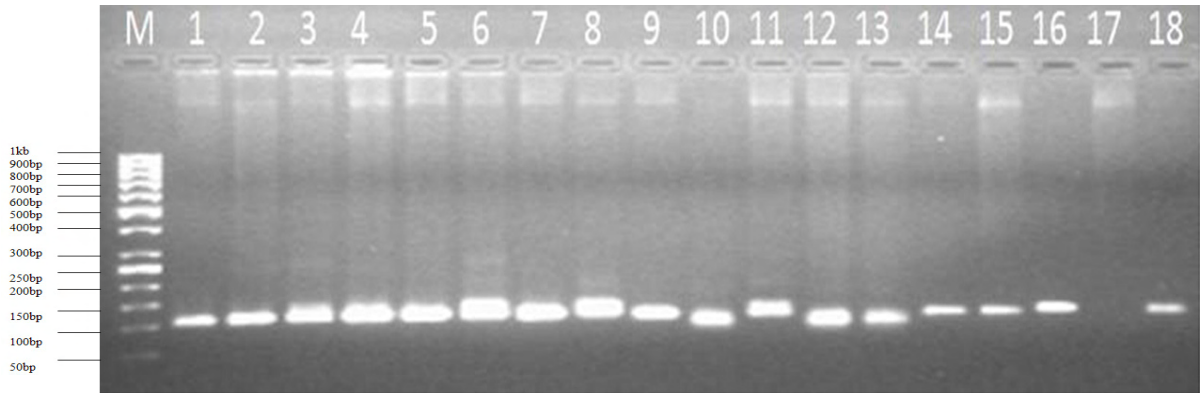

Fig. 1. Amplification of *Xbarc71* microsatellite marker in eighteen wheat cultivars (Lane-M= 50 bp molecular weight ladder; 1: Mons Ald's, 2: HD 2285, 3: Sonalika, 4: Iepaca rabe, 5: AKAW 4008, 6: Halna, 7: Pusa gold, 8: HD 2733, 9: PBW 343, 10: C 306, 11: Kauz/AA/Kauz, 12: Raj 3765, 13: HD 2888, 14: VL 914, 15: F5-995, 16: K0583, 17: SAWSN 3010, 18: Cuo/79/Prulla)

Where, a is number of bands present in cultivars i and j, b is the number of bands present in cultivar i and c is the number of bands present only in cultivar j but absent in cultivar i. The dendrogram was constructed for clustering of wheat cultivars based on matrix of genetic similarities by the unweighted pair group arithmetic mean average (UPGMA) method using the SAHN subprogram of NTSYS-pc 2.1 (Rolf, 1997). The two-dimension principle component analysis (PCA) was also performed for further validation of results of dendrogram. The nature and extent of diversity between cultivars were assessed by identifying the clusters at appropriate similarity coefficient.

Results and Discussion

SSR Polymorphism

To assess genetic diversity among 18 wheat cultivars, 21 microsatellite markers located on 13 different chromosomes with 32 loci were used (Table 3). A total of 273 allelic variants were detected. The number of alleles per locus varied from 5.0 (*Xpsp3071*) to 11.5 (*Xgwm570*) with an average of 8.5 alleles per locus. The ten primer pairs generated amplified products from single locus while remaining 11 primer pairs generated amplified products from two loci.

The genetic diversity at DNA level was estimated using PIC values of microsatellite markers. The highest number of alleles per locus was detected in genome D with 9.3 alleles, compared to 7.3 and 8.6 alleles for genomes A and B (Table 4), respectively. Similar observation of higher polymorphism level in genome D was also reported earlier (Naghavi *et al.,* 2007). This finding was accordance with (Huang *et al.,* 2002), the number of alleles can be used to evaluate genetic diversity because of the significant correlation between gene diversity and the number of alleles. The D genome of *Aegilops squarrosa*, which is the donor of the D genome of common wheat, exhibited more variation than that of common hexaploid wheat (Pestsova *et al.,* 2000; Dudnikov, 2000; Gianibelli *et al.,* 2001). The gene diversity of the B genome was found to be considerably lower than the gene diversity of the genomes A and D. This is consistent with the fact that the B genome is quite different from A and D genomes whereas the A and D genomes are more similar (Bonjean and Angus, 2001). The PIC varied from 0.630 to 0.928 with an average of 0.848 indicating that the markers were highly informative (PIC>0.5). The PIC value in 11 wheat cultivars was reported (Akkaya and Buyukunal-Bal, 2004) in the range of 0.36 to 0.87 with mean of 0.68 using 19 SSR loci. Among the A, B and D genome the mean PIC value were 0.734, 0.888 and 0.857, respectively. The PIC value was higher than the earlier observations (Prasad *et al.*, 2000; Schuster *et al.,* 2009) who reported an average 0.510, 0.710 and 0.490, respectively. The PIC values in Bulgarian winter wheat ranged between 0.100-0.810 (Landjeva *et al.,* 2006).

Cluster Analysis

The genetic relationships among eighteen wheat cultivars were investigated using cluster analysis. Genetic similarity varied from 0.56 (VL 914 Vs. Mons ald's) to 0.97 (Halna Vs. Iepaca rabe and Kauz/AA/Kauz Vs. Halna) indicating a higher level of variation among the cultivars. UPGMA dendrogram divided all wheat cultivars into four main groups (Fig. 2) at 0.81 genetic similarity coefficients. Within the each clusters several sub-cluster were observed and cultivars with more

Table 3. Fragment size, number of loci, number of alleles and polymorphism information content (PIC) in 18 wheat cultivars based on 21 SSR markers

Marker	Fragment size (bp)	No. of loci	No. of alleles	PIC
Xgwm44	140-238	2	22	0.862
Xgwm160	192-252	2	13	0.772
Xgwm190	222-269	1	12	0.928
Xgwm261	178-256	2	14	0.898
Xgwm265	117-194	2	12	0.632
Xgwm272	147-168	1	8	0.841
Xgwm408	161-266	2	12	0.874
Xgwm413	107-161	1	8	0.886
Xgwm437	66-104	1	12	0.922
Xgwm 499	91-151	2	11	0.843
Xgwm513	158-203	1	13	0.916
Xgwm538	167-246	2	18	0.928
Xgwm570	104-206	2	23	0.832
Barc55	138-198	1	15	0.883
Barc75	128-158	1	14	0.922
Wmc341	142-174	1	10	0.910
Wmc397	163-198	1	8	0.910
Xbarc71	125-156	1	11	0.922
Xbarc198	65-174	2	12	0.812
Xgdm132	180-447	2	14	0.630
Xpsp3071	74-208	2	10	0.700
Total		32	273	–
Mean			8.5	0.848

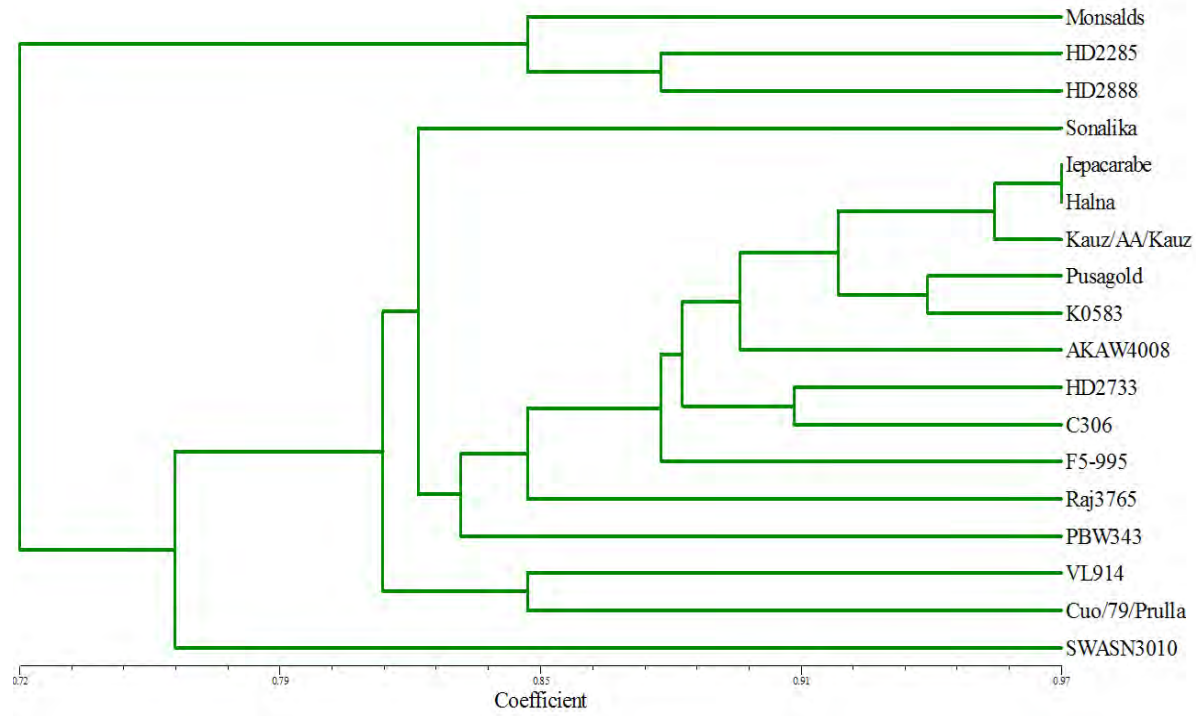

Fig. 2. UPGMA dendrogram showing genetic relationship between 18 cultivars based on Dice similarity coefficient using SSR markers

Table 4. Number of SSR alleles, number of alleles per locus and mean PIC of 32 microsatellite loci in three genomes

Item	Genome			Total
	A	B	D	
Loci	8	14	10	32
Number of alleles	59	121	93	273
Number of alleles/locus	7.3	8.6	9.3	25.2
Mean PIC value	0.734	0.888	0.857	0.826

similarity were placed in the same cluster. In group I, cultivars differentiated into two subgroups comprising, one Mons ald's which is exotic cultivar with lesser similarity on one hand and two Indian cultivars HD 2285 and HD 2888 with higher similarity value (0.88) on the other hand. The larger group II comprised twelve cultivars, can be divided into further two subgroups, subgroup IIA has only one cultivar *i.e.* Sonalika, the most common and widely growing cultivar, while subgroup IIB has eleven cultivars namely, Iepaca rabe, Halna, Kauz/AA/Kauz, Pusa Gold, K0583, AKAW 4008, HD2733, C306, F5-995, Raj3765, PBW343 and most of these cultivars belong to Indian subcontinent. The maximum genetic similarity value (0.97) in this group was observed between Iepaca rabe *vs*. Halna and Kauz/AA/Kauz *vs.* Halna. Cultivars HD 2733 and Sonalika used as check cultivar in many parts of India and in other countries like Bangladesh, Nepal etc. Group III comprise of VL 914 which is an Indian origin cultivar and Cuo/79/Prulla *i.e.* exotic cultivar with 0.84 similarity value and group IV contains only one exotic cultivar, SAWSN 3010 which has different growth type than the other cultivars. Each group consist of similar responsive cultivars with respect to their growth, flowering and maturation time. Cultivars of group II and group III showed maximum similarity while group I and IV showed comparatively less similarity. Among all groups, group I cultivars had completely different growth behaviour than the remaining groups. Results showed that some of the cultivars showing higher similarity of SSR loci were placed in a group, due to similarity of their pedigree from and relative uniformity of their genetic structure. For diversity and population structure analyses different types of marker has been extensively used in wheat, among them most important are Diversity array technology (DArT) (Nielsen *et al.,* 2014) and a single t nucleotide polymorphism markers (SNP) (Ren *et al.,* 2013). In addition, Balestre *et al.* (2008) compared similarity and dissimilarity coefficients using SSR markers data in maize (*Zea mays* L.) and found that the coefficient with the smallest stress value was Dice similarity coefficients. The two-dimensional principle component analysis (PCA) plot was carried out including all cultivars and SSR loci using allele frequency to summarize relationship and validated the results of the dendrogram (Fig. 3). In this study, SSR markers were efficient in discrimination and unambiguous identification of all the eighteen wheat cultivars used in the analysis and information on genetic diversity may be a good tool of selecting genotypes in breeding programs.

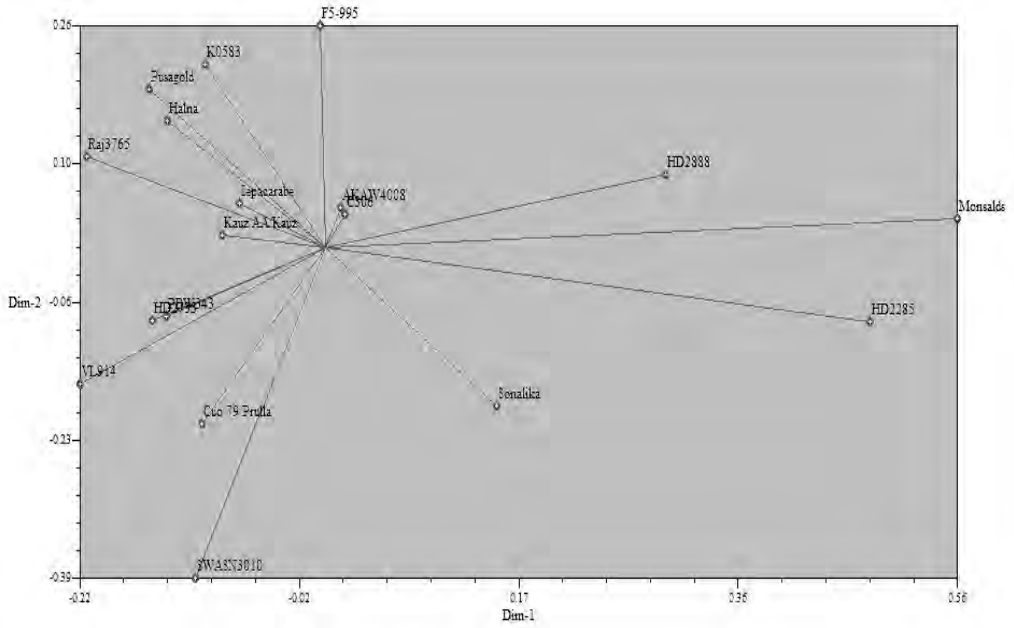

Fig. 3. Scatter plot of the 2-D principal coordinates analyses matrix of SSR marker data.

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Stability of Popular Rice Hybrids for Important Grain Yield Parameters

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Nine popular rice (*Oryza sativa* L.) hybrids were evaluated over three years during *kharif* season 2008- 2010 to assess stability for grain yield and its contributing traits. Analysis of variance of stability revealed highly significant variance due to environment for all seven traits. The variance for genotypic effect was significant for all traits indicating differential response of hybrids over the environments. Estimates of environmental index showed that performance of hybrids over three environments varied and environment E_2 exhibited highest favourable impact on grain yield. Only two hybrids, KRH-2 and PRH-10 revealed stability for grain yield. Hybrid 'KRH-2' showed stable performance for all seven characters whereas, PRH-10 exhibited stability for six traits *viz.*; days to 50% flowering, days to maturity, plant height, panicle length, number of filled grains per panicle and seed yield / m^2. Based on stability parameters these two hybrids can be considered as stable performers and may be exploited for yield enhancement of rice under varying environmental conditions while their parental lines could be used in breeding programme to develop new cultivars with combination of stable characters.

Key Words: G × E interaction, Regression coefficient, Rice hybrids, Stability analysis

Introduction

Rice (*Oryza sativa* L.) is the staple food crop and feeds more than half of the world's population. It plays a vital role in food security and accounts for 30-50 percent of the agricultural income in India (Yadav *et al.*, 2013). India is the second largest rice producing country after China with 105.24 million tons from 43 million hectares of land during 2012-13 (Anonymous, 2014). Despite achieving record rice production, India still needs steady growth to produce 125 million tons of rice by the year 2030 (DAC, 2010). Several varieties have been developed in India but the yield potentials remain stagnant since several years though there has been improvement in quality, disease and pest resistance. Hybrid rice provides an important avenue to achieve higher yields. In China, hybrids accounting for more than half of rice cultivated areas whereas, it is less than 10 percent in India (Spielman *et al.,* 2013). The increase in rice yields in China attributable to the spread of hybrid rice, in turn to, the improved food security for an estimated 60 million additional people per year (Li *et al.,* 2010). Moreover, hybrid rice could be a means of reinvigorating stagnant yield growth in rice in India, boosting rural incomes, and stimulating scientific interest in crop improvement. Widespread adoption of hybrids, therefore is highly depends on their stable high yield performances over the environments. Presently cultivated varieties and hybrids although having high yield potential, they are erratic in their performance even under less varied conditions of cultivation (Saidaiah *et al.*, 2011).

Environmental changes have serious implications on genotypic yield manifestations leads to inconsistency in performance due to genotype × environment interactions (Meena *et al.,* 2014). An understanding of environmental and genotypic causes leading to these interactions is highly important at all stages of plant breeding (Jackson *et al.,* 1996). Allard and Bradshaw (1964) defined stability as adaptation of genotypes to unpredictable and transient environmental conditions. This technique has been used to select stable genotypes unaffected by environmental changes (Das *et al.,* 2010). The method to measure stability was proposed by Finlay and Wilkinson (1963) and later improved by Eberhart and Russell (1966) was used in present study to understand the stability parameters of popular rice hybrids evaluated over three seasons for seed yield and its contributing traits.

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Materials and Methods

The experimental material consisted of nine popular rice hybrids, which were obtained from Indian Institute of Rice Research, Hyderabad. Of these, six hybrids (PRH-10, DRRH-2, KRH-2, CORH-3, Indira Sona and PSD-3) were developed by public sector and three (PA 6129, PA 6201 and PA 6444) were from Bayer Bio-Science (Table 1). Field experiments were carried out at Directorate of Seed Research, Mau located at 25°89' N latitude, 83°46' E longitude and an altitude of 209 feet above mean sea level under North Eastern Plains Zone (NEPZ) in the fertile plains of the Ganges–Ghaghara doab. The trials were conducted over three years from 2008 to 2010 during *Kharif* at same site in successive seasons representing three different environments E_1 (2008), E_2 (2009) and E_3 (2010), respectively. The average rainfall was 800 mm of which more than 80 per cent was received during the monsoon. Soil of experimental plots was silty loam having 55.9, 31.0 and 13.1 per cent proportions of sand, silt and clay, respectively, and slightly alkaline with pH ranging from 7.5 to 8.1. The organic carbon, available N, P and K were 0.32 per cent, 206 kg/ha, 19.7 kg/ha and 215 kg/ha, respectively. Experiments were laid out in randomized complete block design (RCBD) with 3

Table 1. Details of rice hybrids utilized for the study

Name	Year of release	Developed by	Area of adoption	Characteristics of hybrid
PRH-10 [S]	2001	Indian Agricultural Research Institute, New Delhi	Delhi, Haryana, Uttar Pradesh, Uttara Khand	Aromatic basmati hybrid, early maturity (115-120 days), high yielding (50-60q/ha), fine grain, high milling recovery, milled aroma, good cooking quality, enough volume expansion, non-glutinous and good taste.
DRRH-2[S]	2005	Directorate of Rice Research, Hyderabad	Haryana, Tamil Nadu, Uttara Khand, West Bengal	Suitable for irrigated conditions, semi dwarf (90 cm), early maturity 116 days, slender grains, resistant to lodging, LF, neck blast and RTV, moderately resistant to Sh.RBS & WBPH, and good yield (54 q/ha).
KRH-2 [S]	1996	VC Farm, (ARS of UAS, Bangalore), Mandya	Bihar, Haryana, Karnataka, Maharashtra, Odisha, Pondicherry, Rajasthan, Tamil Nadu, Tripura, Uttara Khand, West Bengal	Suitable for irrigated timely sown, maturity125-130 days, semi dwarf (102 cm), grains long slender and white, tolerant to LB, BS, & other diseases and high yielding (75-85 q/ha).
CORH-3[C]	2006	Tamil Nadu Agricultural University, Coimbatore	Tamil Nadu	Suitable for aerobic cultivation and mid-season drainage, early maturing (115days), high yielding (72 q/ha), grains medium slender and white, non-sticky, non-aromatic rice and good taste, tolerant to Rice Tungro Disease (RTD) and blast, resistant to Green Leaf Hopper (GLH) and tolerant to Brown Plant Hopper (BPH) and White Backed Plant Hopper (WBPH).
Indira Sona[S]	2006	Indira Gandhi Krishi Vshwa Vidyalay, Raipur	Chhattisgarh	Suitable for shallow lowlands, mid-early (125-130 days), high grain yield (60-65 q/ ha), grains long slender, mild aroma, resistant to gall midge and tolerant to blast.
PA 6129[S]	2007	Bayer Bio-Science, Hyderabad	Pondicherry, Punjab, Tamil Nadu	Early maturity (120 days), semi-dwarf, grains long slender, better N responsive, suitable to intermittent drought conditions, resistant to leaf blast, high yielding (60 – 80 q/ha).
PA 6201[S]	2000	Bayer Bio-Science, Hyderabad	Andhra Pradesh, Bihar, Karnataka, Odisha, Madhya Pradesh, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal	Suitable for irrigated conditions, mid-early (125-130 days), good yield (62 q/ha), grains long bold, resistant to blast, tolerant to SB, BPH & LF.
PA 6444 [S]	2001	Bayer Bio-Science, Hyderabad	Andhra Pradesh, Karnataka, Maharashtra, Odisha, Tripura, Uttar Pradesh, Uttara Khand	Suitable for irrigated conditions, matures in 135-140 days, semi tall (100-120 cm), compact and erect, medium slender grains and good yield (60-65 q/ha).
PSD-3[C]	2004	Govind Ballabh Pant University of Agriculture &Technology, Pantnagar	Uttara Khand	Suitable for irrigated plain areas, mid-early (125-130 days), fertilizer responsive and non lodging, tolerant to salinity & alkalinity, long slender grains with mild aroma, moderately resistant to BB, stem borer and BHP, yield 60-65 q/ha.

[S] State Release Hybrid; [C] Central Release Hybrid

replications in plot size of 5 × 3m during each season. The 21 days old seedlings were transplanted keeping plant to plant and row to row spacing of 20 and 15 cm, respectively. All recommended cultivation practices prescribed for rice were followed precisely.

Observations were recorded from each plot for seven metric traits, *viz*. days to 50% flowering, days to maturity, plant height (cm), panicle length (cm), number of effective tillers per plant, number of filled grains per panicle and grain yield/m^2 (g). The data on grain yield was recorded on per m^2 basis, while, days to 50 % flowering was recorded on plot basis. Observations on remaining characters were recorded on ten randomly selected competitive plants from each plot of every replication. Standard procedures for analysis of variance were followed. Data were first subjected to the analysis followed for randomized block design as per Panse and Sukhatme (1967). Stability analysis was carried out following Eberhart and Russel (1966). Estimates of mean performance (x), regression coefficient (bi) and deviations from regression (S^2di) were used to draw inferences on stability of different rice hybrids.

Results and Discussion

Analysis of variance revealed significant differences amongst genotypes in each of the three environments. The significant variance due to environment and genotypes for the traits indicated distinct and differential effect of environments and differential response of genotypes (Table 2). Similarly, the variance due to environment + (genotype × environment) interactions was highly significant for all the traits interacted that genotypic and environment interaction was prominent. The findings are similar to those reported earlier by several researchers (Deshpande and Dalvi, 2006; Ramya and Senthil, 2008; Saidaiah *et al*., 2011). Highly significant variation due to environment (linear) was observed for days to 50% flowering, days to maturity, plant height, panicle length, number of effective tillers per plant, number of filled grains per panicle and seed yield / m^2 revealed linear contribution of environmental effects and additive environmental variance on these characters. Similar results were also reported by Lavanya *et al.* (2005), Deshpande and Dalvi (2006), Arumugam *et al.* (2007) and Saidaiah *et al*. (2011). Genotype × environment (linear) interaction was significant for days to maturity and plant height suggesting that genotypes differ for their linear response to environments. The pooled deviation was significant for five characters indicating that some portion of G × E interaction was unpredictable. Significant non-linear responses were earlier reported by Babu *et al.* (2005), Bhaktha and Das (2008) and Johnson *et al.* (2010), whereas both significant and non-significant linear responses were observed by Lavanya *et al.* (2005) and Vidhu Francis *et al*. (2005).

The estimates of environmental index can provide the basis for identifying favourable environments for expression of maximum potential of the genotype (Breeze, 1969). The comparison of environmental indices (Table 3) indicated that the performance of nine rice hybrids over three environments (cropping seasons) with respect to the grain yield / m^2 varied apparently and environment E_2 (*Kharif* 2009) exhibited highest favourable impact on grain yield followed by E_3 (*Kharif* 2010). Moreover, number of filled grains / panicle were higher under E_2 (*Kharif* 2009) while, favourable impact

Table 2. Analysis of variance for stability of important yield traits in rice hybrids

Source of variation	DF	Days to 50% flowering	Days to maturity	Plant height (cm)	Panicle length (cm)	Number of effective tillers / plant	Number of filled grains / panicle	Grain yield / m^2 (g)
Genotype	8	93.07**	72.40**	212.41**	3.20*	1.53*	969.82**	7461.95*
Environment	2	102.52**	67.98**	299.27**	21.42**	4.83**	2582.81**	45876.13**
Genotype x Environment	16	1.73	1.69	21.65	0.645	0.51	79.38	3006.06
Environment + (Genotype x Env)	18	12.93**	9.05**	52.49**	2.95*	0.99*	357.54**	7769.36*
Environment (linear)	1	205.04**	135.97**	598.55**	42.83**	9.67**	5165.63**	91752.27**
Genotype x Environment (linear)	8	2.05	2.59*	33.51*	0.42	0.69	87.29	3828.12
Pooled deviation	9	1.25**	0.69*	8.71**	0.77	0.28	63.52**	1941.25**
Pooled error	48	0.25	0.313	2.94	0.41	0.17	17.27	34.72

*Significant at P=0.05; **Significant at P=0.01

Table 3. Effect of environment in the expression of grain yield and contributing traits

Character	Environmental indices		
	E_1	E_2	E_3
Days to 50% flowering	-3.765	1.012	2.753
Days to maturity	-3.173	1.531	1.642
Plant height (cm)	-4.182	6.578	-2.397
Panicle length (cm)	1.391	0.268	-1.659
Number of effective tillers / plant	-0.119	-0.667	0.785
Number of filled grains/ panicle	-19.556	10.185	9.370
Grain yield /m^2 (g)	-78.346	61.395	16.951

of effective tillers / plant was pronounced in environment E_3. Environment E_1 (*Kharif* 2008) was observed to be unfavourable in terms of grain yield; however, hybrids during this season exhibited early maturity and shorter plant height, while most of the traits were in lower side as indicated by negative values of environmental indices. The results are in agreement with the earlier findings of Babu *et al.* (2005), Sedghi-Azar *et al.* (2008) and Saidaiah *et al.* (2011).

According to Eberhart and Russel (1966), a stable genotype is one that shows higher mean yield, regression coefficient equal to unity (bi=1) and mean square deviation from regression near to zero ($S^2di=0$). Linear regression (bi) is a measure of response to environmental changes of a genotype whereas, deviation from regression measures the stability of genotypes with lowest standard deviation near to zero being the most stable and *vice-versa*. In interpreting the results, deviation from regression (S^2di) was considered as the measure of stability as suggested by Breeze (1969). Then, the measure of response to environmental changes (type of stability) was decided on regression coefficient (bi) and mean values (Finlay and Wilkinson 1963). With respect to plant height, days to 50% flowering and days to maturity the mean values lower than population mean were considered as desirable while, higher mean values were considered as desirable for remaining traits.

The estimates of stability parameters for seven characters presented (Table 4) revealed that hybrids, PRH-10, KRH-2 and PA 6201 possessed minimum deviation from regression (S^2di) indicating their high predictability in terms of grain yield /m^2. The hybrid KRH-2 possessed higher grain yield, non-significant regression coefficient (bi) less than one and with predictable performance over three seasons was considered to be stable and ideal for unfavourable environments. The stability parameters for hybrid, PRH-10 revealed non-significant S^2di values and regression coefficient (bi) with mean yield of 579.1g which was slightly lower than population mean (611.01g), and higher regression coefficient indicated that its suitability for favourable environments. Lavanya *et al.* (2005), Panwar *et al.* (2008) and Saidaiah *et al.* (2011) recorded variable response and specific adaptability of rice hybrids. Similarly, Bhakta and Das (2008) also reported average stability of rice genotypes with low yield potential, unit regression and non-significant S^2di values. Thus, the results shows that genotypes with moderate productivity can exhibit wide adaptability over range of environments and high yielding genotypes that are brought about by genetic manipulation may lead to variable performances. It is therefore, necessary to combine these two important genetic traits *viz.*; high yield potential and greater stability in the development of superior hybrids / genotype (s) in rice. Madariya *et al.* (2001) emphasized that grain yield is a complex trait and the analysis of individual yield parameter may lead to simplification in explaining stability for grain yield. Hence, it was observed (Table 4) that KRH-2 was the only hybrid showed stable performance for all seven characters with non-significant regression coefficient and S^2di values although, it was slightly late in maturity (128.2) and taller in height (131.4cm) compared to population means of 125.6 and 116.1, respectively. Hybrid PRH-10 exhibited stability for six traits, including days to 50% flowering, days to maturity, plant height, panicle length, number of filled grains per panicle and seed yield / m^2. Thus, the stability for grain yield in these hybrids (KRH-2 and PRH-10) was compensated by stability of different yield contributing parameters, resulted into wider adaptability of the hybrids.

All hybrids except DRRH-2 and PSD-3 showed linear predictability with non-significant deviation from regression (S^2di) for days to 50% flowering. Two hybrids, CORH-3 and PA 6129 possessed desirably lower mean values and non-significant regression coefficient (bi) greater than unity, hence were stable and suitable for favourable environments whereas, hybrid PRH-10 having regression coefficient less than unity with desirable mean was stable and suitable for unfavourable conditions. For days to maturity, six hybrids exhibited non-significant deviation from regression (S^2di) signifies their linear predictability. Among the hybrids, CORH-3 revealed stability and suitability for favourable environments

while, two hybrids PRH-10 and PA 6129 observed to be stable and ideal for unfavourable conditions. Similarly, with respect to plant height, seven hybrids showed linear predictability and out of them four hybrids namely, PRH-10, Indira Sona, PA 6129 and PSD-3 had desirable mean values, non-significant regression coefficient (bi) greater than one showed stability and were suitable for favourable environments.

The stability parameters for number of effective tillers / plant, in eight hybrids indicated predictable S^2di values of them four hybrids *viz*; DRRH-2, KRH-2, CORH-3 and Indira Sona possessed higher mean values. KRH-2 and Indira Sona had regression coefficient greater than one while, DRRH-2 and CORH-3 had less than one meaning thereby that these hybrids were stable and ideal for favourable and unfavourable environments, respectively. Similarly, with respect to number of filled grains / panicle, six hybrids showed linear predictability with non-significant deviation from regression (S^2di) as per stability criteria's, hybrid KRH-2 revealed stability under favourable environments and hybrids PRH-10 and PA 6129 under poor environments.

Genotype × environment (GE) interaction for grain yield in rice has been reported in numerous studies under several designations like different response patterns, adaptation, or stability of a genotype, *etc*. The adaptability of a genotype to a range of environments can be best explained by its phenotypic stability (Elfadl *et al.*, 2012). This concept of stability implies that a stable variety may not necessarily respond to better growing conditions with increased yield (Becker, 1981). Identification of yield-contributing traits and estimation of genotype × environment (GE) interaction, and yield stability are the important parameters for breeding new cultivars adapted to specific environmental conditions (Rao *et al.*, 2002). Selection of genotypes for adaptability could be severely limited in terms of GEI. Therefore, it is necessary to assess the environmental sensitivity of genotypes in terms of higher yield and stability (Thillainathan and Fernandez, 2001). The hybrids with specific adaptability (favourable/poor environments) rather than general might overcome the problem of genetic vulnerability (Saidaiah *et al.*, 2011).

Based on stability criteria's *i.e.* regression coefficient (bi values), deviation from linearity (S^2di values) and mean performance, some of the hybrids were identified with stable performance under favourable and some under poor environments in terms of grain yield and contributing attributes (Table 5). The stability of various contributing traits varied in compensating manner in different hybrids imparted grain yield stability. The hybrids exhibited stable yield performance could be exploited for general cultivation. The parental lines of such hybrids may be used to develop new diverse strains with combination of stable characters. In nut shell, two hybrids KRH-2 and PRH-10 have shown higher mean values, desirable regression coefficient and deviation from the regression coefficient for yield and contributing traits, and were good in yield can be exploited for yield enhancement of rice under varying environments.

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Table 4. Mean performance and stability parameters for yield parameters of rice hybrids over environments

	Days to 50% flowering			Days to maturity			Plant height (cm)			Panicle length (cm)			Number of effective tillers / plant			Number of filled grains / panicle			Grain yield / m^2 (g)		
	μ	βi	σ^2di	μ	βi	σ^2di	μ	βi	σ^2di	μ	βi	σ^2di	μ	βi	σ^2di	μ	βi	σ^2di	μ	βi	σ^2di
PRH 10	83.44	0.89	-0.06	119.67	0.74	0.94	106.38	1.07	1.99	28.19	1.66	1.23	8.76	1.82	0.65*	155.22	0.85	-9.15	579.11	1.40	88.80
DRRH 2	86.11	0.88	4.20**	122.11	0.77*	-0.31	111.48	0.21*	-2.94	28.84	1.20	-0.36	10.76	0.38	0.18	115.55	1.12	-10.52	546.44	1.26	4733.62**
KRH 2	92.22	0.95	-0.16	128.22	1.12	-0.31	131.48	1.47	-3.00	29.23	0.96	1.17	9.74	1.09	0.01	161.88	1.15	-13.66	655.22	0.66	2.81
CORH 3	84.00	1.46	0.19	121.88	1.43	1.35*	114.78	0.71	34.23**	28.69	1.00	0.93	10.09	-0.61	0.03	145.00	1.34	-11.96	628.66	0.13	764.29**
Indira Sona	91.78	0.49	-0.10	128.11	1.19	-0.31	115.10	1.81	-0.35	29.65	0.80	0.37	9.43	1.16	-0.04	139.67	1.50	207.23**	528.11	0.70	9408.44**
PA 6129	82.67	1.39	-0.12	120.22	0.39	1.00*	106.09	1.22	-1.55	26.17	0.97	-0.37	9.01	0.69	-0.04	169.22	0.48	0.63	657.55	1.40	590.56**
PA 6201	92.11	1.08	-0.19	128.44	1.50	1.36*	121.35	0.54	3.19	27.91	0.63	-0.32	8.98	1.53	0.16	162.33	0.36	218.20**	641.88	0.77*	31.14
PA 6444	99.44	0.77	0.28	134.22	0.49*	-0.31	124.98	-0.05	19.83**	28.89	0.97	-0.27	8.51	2.06	-0.01	168.33	0.88	51.75*	659.78	0.52	286.09**
PSD 3	91.77	1.04	5.19**	128.33	1.36	0.04	113.90	2.04	-0.11	29.22	0.80	0.68	9.04	0.88	-0.01	134.44	1.31	-2.91	602.33	2.16	1345.17**
Pop.mean	**89.28**			125.69			116.17			28.53			9.37			150.18			611.01		
SE (mean)	0.79			0.60			2.10														
SE (bi)	0.23			0.20			0.40														

*Significant at P=0.05; **Significant at P=0.01

Table 5. Stability of rice hybrids over different environments for grain yield and yield attributes

	Hybrids		
Character	Average stability	Below average stability (suitable for favourable environment)	Above average stability (suitable for unfavourable environment)
Days to 50% flowering	-	CORH-3, PA 6129	PRH-10
Days to maturity	-	CORH-3	PRH-10, PA 6129
Plant height (cm)	-	PRH-10, Indira Sona, PA 6129, PSD-3	-
Panicle length (cm)	CORH-3	DRRH-2	KRH-2, Indira Sona, PA 6444, PSD-3
Number of effective tillers/ plant	-	KRH-2, Indira Sona	DRRH-2, CORH-3
Number of filled grains/ panicle	-	KRH-2	PRH-10, PA 6129
Grain yield /m^2 (g)	-	PRH-10	KRH-2

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Seed Protein Profiling of Tomato (*Solanum lycopersicum* L.) Genotypes using SDS-PAGE

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A quantitative categorization of seed storage proteins profiles of 13 genotypes of *Solanum lycopersicum* L. was performed by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The banding patterns were characterized by 3 clear distinct zones viz., A, B and C. The unweighed pair group method using arithmetic average (UPGMA) analysis of 13 tomato genotypes was done and two major clusters obtained through seed protein analysis expressed better grouping of genotypes. The dendrogram showed that the genotype EC-519724 was most dissimilar from other genotypes.

Key Words: Cluster analysis, Germplasm, SDS-PAGE, *Solanum lycopersicum* L, Tomato

Tomato ((*Solanum lycopersicum* L.) popularly known as 'Love Apple' is one of the most widely grown and commercially important vegetable crops. Tomato is one of such crop which has received wider attention of vegetable breeders in various countries. Lot of diversity is found in growth and development pattern of tomato due to altitudinal variation in Uttarakhand. To conserve the genetic diversity, elucidation of genetic diversity is extremely necessary for the effective maintenance, evaluation and utilization of germplasm because it is the only source to be exploited for the development of new varieties during breeding programs. The problem of cultivar identification has been simplified to a great extent by the combined use of morphological, biochemical and molecular markers. Morphological markers are highly influenced by environment (Goodrich *et al*., 1985). The biochemical markers are proteins that can be isolated and their polymorphism identified through electrophoresis. Biochemical markers such as proteins and isozymes have served as important tools to detect genetic relationships in plants (Mukhlesur *et al.*,2004; Erum *et al*., 2011). The electrophoresis of proteins is a method to investigate genetic variation and to classify plant varieties (Isemura *et al.*, 2001). Its banding pattern is very stable and it is advocated for cultivars identification purpose in crops. It has been widely accepted that such banding patterns could be important supplemental method for cultivars identification (Tanksley and Jones, 1981; Thanh and Hirata, 2002). Numerous electrophoresis methods are available to identify cultivars by protein banding patterns. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) provides the best resolution. Analysis of SDS-PAGE is simple and inexpensive, which is an added advantage for use in practical plant breeding. Therefore, present study was undertaken to characterize 13 genotypes of Tomato through seed Protein banding pattern.

Seeds of 13 genotypes were procured from Pantnagar Centre for Plant Genetic Resources of G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The SDS-PAGE is used for seed protein profiling as described by Laemmli, 1970 with slight modification. Each sample having 0.1g seed were crushed in 1 ml extraction buffer (1 M Tris-HCl pH 8.0, 2% SDS, 10% glycerol, 1mM PMSF- phenyl methyl sulphonyl fluoride and 2% mercaptoethanol). The mixture was heated at 65°C for 20 minute and then centrifuged it for 20 minute at 10,000 rpm at room temperature. The supernatant was collected and stored at 4°C for further use. Equal volume (20 µl) of supernatant i.e. protein sample and sample buffer were mixed. Then, it was heated at 60°C in water bath for 5 min. and after cooling samples were loaded to each well along with marker protein in one well with help of micro syringe. The SDS solubilised protein samples were then subjected to vertical slab SDS-PAGE with 12.5% separating gel and 5% stacking gel using Tris-glycine electrode buffer. The samples were electrophoresed at 80 V initially and

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increased upto 100 V with current 500 mA, when the tracking dye passed from the stacking gel. The run was stopped when the dye was approximately 0.5 cm away from the bottom of the gel, which took around 6 to 7 hours. The gel was removed with the help of spatula and dipped for overnight in staining solution. (0.2% coomassie brilliant Blue R 250, 60 g TCA, 180 ml methanol and 60 ml glacial acetic acid). Next day, destaining was performed; the gel was observed on Syngene Gel Documentation system and photographed. Protein bands were scored for their presence as 1 and absence as 0 from the de-stained gel. Presence and absence of the bands were entered in a binary data matrix. Based on results of electrophoretic band spectra, similarity index was calculated for all possible pairs of protein type's electrophoregrams. Coefficient of similarity were calculated by using Jaccard's similarity coefficient by SIMQUAL function and cluster analysis was performed by UPGMA(unweighed pair group method using arithmetic average) method by SAHN clustering function of NTSYS-PC(Numerical taxonomy and Multivariate analysis System programme) version 2.0 (Rohlf, 1987)

Seed Storage proteins are highly independent of environmental fluctuations. The high stability of seed protein profile and its additive nature makes it a promising tool for distinguishing genotypes of particular plant species. Therefore, in the present study an attempt has been made to give a blue print of the genetic diversity of genotypes of tomato through SDS-PAGE technique. The seed protein fragments (Plate1) exhibited appreciable polymorphism amongst the thirteen genotypes used for study and the diagrammatic representation has been depicted in Zymogram (Fig. 1). The degree of variation in the bands is interpreted as a measure of genetic divergence among cultivars (Siddiqui and Naz, 2009).

A total of 12 protein bands were obtained which were further categorized under 3 distinct Zones A, B, C depending on their decreasing molecular weight and increasing Rf values. The high molecular weight proteins were located in upper region and low molecular weight protein in the lower region of the gel. A standard medium range protein marker of known molecular weight (14.3 KD to 97.4 KD) was used along with samples. The protein bands were stacked according to their molecular weight i.e. high molecular weight proteins were located in upper region and low molecular weight proteins in the middle and lower region of the gel, respectively. Different protein electrophoretic pattern exhibited by the cultivars could be identified solely by the cultivar specific. For genotype discrimination the presence and absence of protein bands was the criteria for characterisation of germplasm differentiation.

Among 12 bands maximum number of bands was observed in PT-09-06 (10 Bands), EC-519724 (10

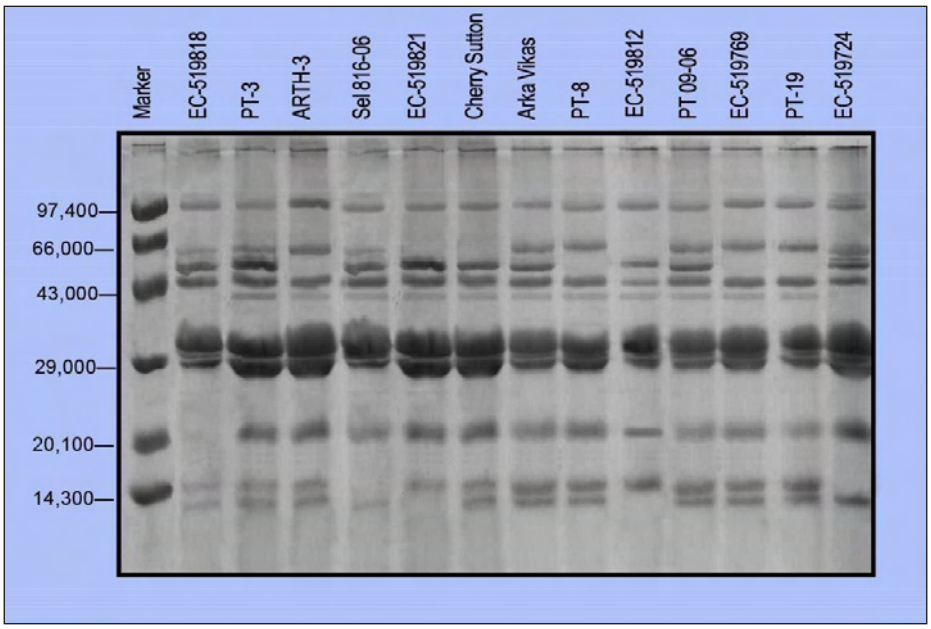

Plate 1. Protein profiling of Tomato through SDS-PAGE

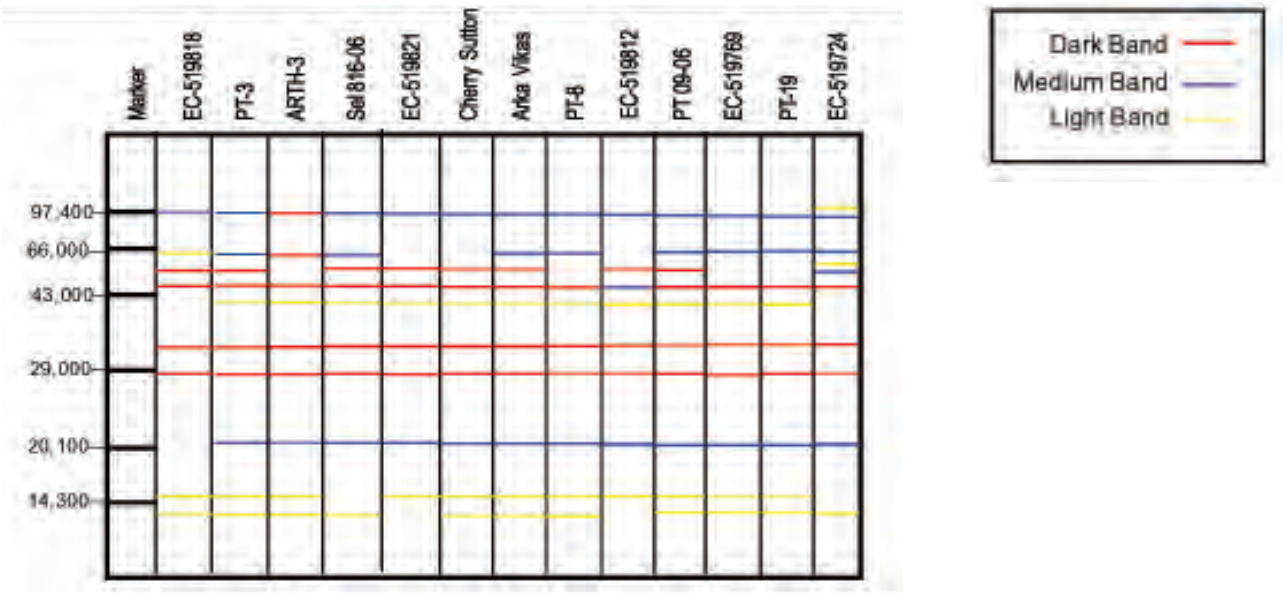

Fig. 1. Protein profiling of Tomato through SDS-PAGE

bands), Arka vikas (10 bands) and PT-3 (10 bands). In contrast to this result, Vishwanath *et al.* (2011) reported that Arka Vikas showed 14 bands.

Zone A representing the heaviest molecular weight protein ranges from 97 KDa to 43 KDa was subdivided into 7 distinct bands. The Subzone A1 was present only in EC-519724. The subzone A2 was present in all genotypes. The subzone A3 were present in ten genotypes and this zone was found absent in EC-519821, EC-519812 and Cherry Sutton. The subzone A4 was present only in EC-519724. The subzone A6 was present in all genotypes. Zone B representing mostly thick and dark bands subdivided into 2 bands i.e. B1 and B2. Bands B1 and B2 were present in all genotypes. The Zone C comprised of 3 Medium and light bands. These were designated as subzones C1, C2 and C3. The subzone C2 were present in 11 genotypes and this was found absent in Sel-816-06 and EC-519724. The subzone C3 present in 11 genotypes and this was found absent in EC-519821 and EC-519812 .Thus a total of 12 bands could be resolved across thirteen genotypes of tomato.

The cluster analysis distinguishes genotypes on the basis of their diversity and could be used as basis of selection of genotypes for crop improvement (Bharose *et al*., 2014). Cluster analysis (Fig. 2) after quantifying the protein bands using UPGMA procedure, indicated that broadly the genotypes were grouped into two major cluster at about 68% similarity index. The major cluster was further divided into two sub-clusters with approximately 78% and 88% similarity. Genotype namely Cherry Sutton showed 100% similarity with other genotypes of same sub-cluster namely EC 519821and EC-519812 in one group which expressed 100% genetic similarity within group. These genotypes were morphologically distinct with each other. The dendrogram showed that the genotype EC-519724 was most dissimilar from other genotypes. As a group genotype EC-519818 was dissimilar from that of PT-3, Arka Vikas, PT-09-06, ARTH-3, PT-8, E-519769, PT-19, Sel-816-06, EC-519821, EC-519812 and Cherry Sutton. Genotypes PT-3, Arka Vikas and PT-09-06 were related and formed one group. Another such group of related genotypes included ARTH-3, PT-8, EC-519769 and PT-19 was also related and formed the second group. Next similarity group was constituted by EC-519821 and EC-519812. One genotype as independent namely EC- 519724 was found most diverse among all genotypes and showed low similarity 75 % with the other genotypes of clusters.

Broadly, these clustering of genotypes to assess genetic diversity in tomato germplasm have also been reported (Hady *et al.*, 2010). The phylogenetic evolutionary tree developed from the analysis indicated that most of the tomato genotypes did not form distinct clusters; rather, the relatively close and small clusters of genotypes were distributed within one broad cluster as can be seen in the phenogram obtained in cluster 1 with 6 groups of 12 genotypes of tomato. The clustering

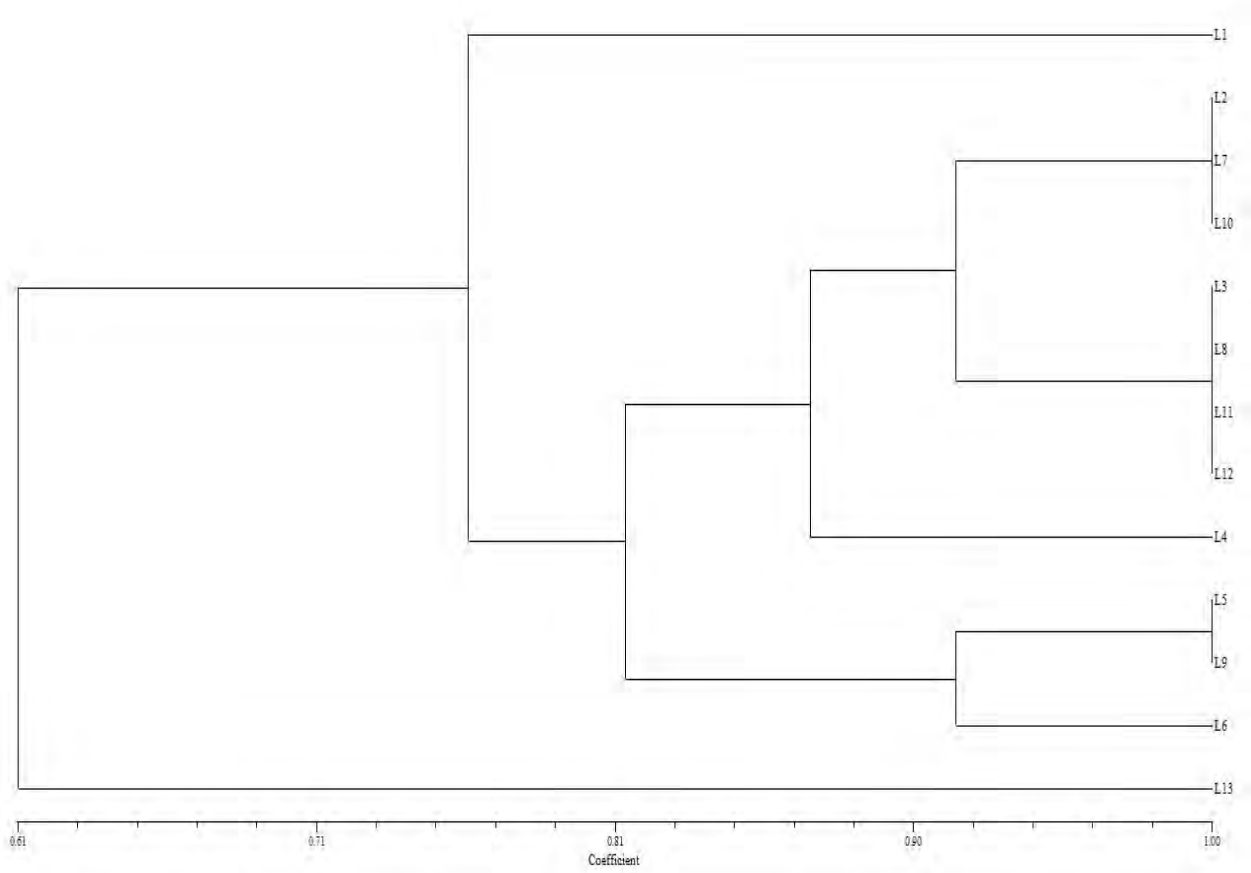

Fig. 2. Dendrogram of thirteen tomato genotypes, L1=EC-519818, L2=PT-3, L3=ARTH- 3, L4=Sel-816-06, L5=EC 519821, L6= Cherry Sutton, L7=Arka Vikas, L8= PT- 8, L9=EC-519812, L10= PT-09-06, L11= EC-519769, L12= PT-19, L13=EC- 519724

pattern indicates though, genotypes are morphologically different but at the genotypic level they are close to each other.Thus, the seed protein profiling emerges as a potent technique to generate wide array of polymorphism and as such it could serve valuable information for varietal identification and extent of genetic diversity. Further, an integrated approach utilizing both morphological and biochemical markers could help in proper characterization of tomato germplasm.

It's concluded from the study that EC 519818 and EC 519724 are most diverse among all genotypes and may be further utilized as potent genotypes in tomato breeding programme for the varietal development.

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Genetic Variability and Genotypic × Environment Interaction for Seed Yield and Related Traits in French Bean Germplasm

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Thirty-three accessions of bush type French bean were evaluated in Randomized Complete Block Design with three replications for two seasons during summer 2008 and 2009 at Vegetable Farm, H.P. Agricultural University, Palampur to explore the genetic variability and G × E interaction for yield and yield related traits. High genotypic and phenotypic coefficients of variations were observed for seed yield/plant followed by moderate for majority of the traits. A positive correlation was observed for seed yield/plant with fresh pod yield/plant, pods/plant, 100-seed weight, average pod weight, plant height, pod length, biological yield/plant and branches/plant. On the basis of correlation and path coefficient analysis, selection on the basis of pods/plant, fresh pod yield/plant, 100-seed weight, plant height and branches/plant would be a paying proposition for evolving high yielding genotypes. A significant association between seed yield/plant and fresh pod yield is of significance to explore the possibilities to develop dual purpose French bean variety(ies) both for vegetable and dry bean.

Key Words: Attributes, Correlation, Genetic variation, Path analysis, *Phaseolus vulgaris* L., Yield

Introduction

French bean (*Phaseolus vulgaris* L.), being legume crop, is an important source of protein, calcium, iron and vitamins in human diet. It finds very coveted position in the hill states of India for its off-season cultivation and quality pods which in turn results in high remuneration to the vegetable growers. The age old variety 'Contender' is still cultivated by the farmers due to lack of suitable cultivars with desirable horticultural traits. The variety is prone to plethora of diseases and has curved pods which have the tendency to break in transit and thereby, results in heavy losses. So, genetic restructuring of French bean germplasm is required to develop high yielding varieties with desirable traits. The improvement potential of any crop is proportional to the magnitude of genetic variability in the germplasm (Singh *et al.* 2009) and a wide range of variability for various traits is available in *Phaseolus* spp. This provides the possibility to improve the yield and quality through strategic breeding programme. Selection of one trait invariably affects number of associated traits, which evokes the need to find out the interrelationship of various yield components among themselves and with yield. The knowledge of the interrelationship between different traits is important in breeding for direct and indirect selection of traits that are not easily measured and have low heritability. The consistent association of yield characters over environment is of immense importance to explore the breeding efficiency (Adunga and Labuschangne 2003). Therefore, it is necessary to examine the magnitude of correlation between characters for achieving the higher yield. However, the correlation studies do not provide the exact picture of relative importance of direct and indirect effects of each of the component character. Moreover, when more and more variables are included, the indirect association becomes more complex. Under such situations, path coefficient analysis helps in partitioning the correlation into direct and indirect effects. Keeping this in view, a study was undertaken to analyse the relationship between yield components, association amongst the desired traits and their direct and indirect contribution toward the seed yield in 33 potential genotypes of bush type french bean. The information shall enable the breeders to make informed decisions about suitable parents while planning french bean breeding programme for high yield along with desirable horticulture traits.

Materials and Methods

The experiment was undertaken at the experimental farm of the Department of Vegetable Science and Floriculture,

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CSK HPKV, Palampur (1290.8 m amsl, with latitude 32^0 6' N, longitude 76^0 3' E). The 33 genotypes (Table 1) were laid in Randomized Complete Block Design for two seasons during March-June 2008 and 2009. The sowings were done at spacing of 0.45m between rows and 0.15m between plants within rows in two rows of 2.7m length. Data were recorded on ten competitive plants taken at random in each entry for twelve yield and other related traits *viz*., days to 50% flowering, average pod weight (g), branches/plant, pods/plant, fresh pod yield/plant (g), days to seed maturity, pod length (cm), seeds/pod, biological yield/plant (g), plant height (cm), 100-seed weight (g) and seed yield/plant (g).

The crop was well managed for optimum growth and yield. The seeds were sown at a depth of 4-5 cm. The fertilizers were applied at the time of sowing @ 50 kg N: 90 kg P_2O_5 and 60 kg K_2O/ha. Weeds were controlled with Pendimethalin @ 1 kg a.i./ha as pre-emergence application at the initial stages of growth followed by two manual weedings at 40 and 60 days after sowing. The irrigation was applied at 15 days interval depending upon the requirement.

Analysis of variance was performed for individual season and error variance was tested for homogeneity (Gomez and Gomez 1983). The combined analysis of variance of two season's data was done for each trait for 33 French bean accessions. The genotypic, phenotypic and environmental coefficients of variations were estimated following the method of Burton and De Vane (1953). Heritability and expected genetic advance (GA) were calculated as per Burton and De Vane (1953) and Johnson *et al.* (1955), respectively. Coefficients of correlation were calculated as suggested by Al-Jibouri *et al.* (1958) and path coefficients of different traits with seed yield /plant were carried out as per the method of Dewey and Lu (1959).

Results and Discussion

Seasons were significantly different for majority of the traits except number of branches/plant, pods/plant, pod length and seeds/pod. This indicates that these traits were more stable than the other across the seasons (Table 2). This uniformity is very useful for selection of parents for high pod yield. The French bean genotypes were significantly different for almost all the characters and there were significant genotypic and environmental interactions for these yield and related traits except for branches/plant, pod length and seeds/pod. The results showed genetic variability for these characters and also indicated the need for multi-location trials to identify the superior genotypes. The contribution of G×E interaction was lower than the contribution of genotypic differences in all the traits. A wide range in means (Table 3) of the

Table 1. List of genotypes used in the experiment and their source

S. No.	Genotypes	Source	S. No.	Genotypes	Source
1	Arka Suvidha	IIHR, Hasarghata	18	KPV-2	CSKHPKV, Palampur
2	Arka Anoop	IIHR, Hasarghata	19	MFB-1	NEH, ICAR, Barapani
3	DPDFB-1	CSKHPKV, Palampur	20	MFB-2	NEH, ICAR, Barapani
4	DPDFB-1(M)	CSKHPKV, Palampur	21	MFB-3	NEH, ICAR, Barapani
5	DPDFB-2(M)	CSKHPKV, Palampur	22	MFB-4	NEH, ICAR, Barapani
6	DWDFB-I	UAS, Dharwad	23	MFB-5	NEH, ICAR, Barapani
7	DWDFB-53	UAS, Dharwad	24	VLB-8	VPKAS, Almora
8	DWDFB-57	UAS, Dharwad	25	VLB-9	VPKAS, Almora
9	HAFB-1	HARP, Ranchi	26	VLB-2003	VPKAS, Almora
10	HAFB-2	HARP, Ranchi	27	VLFB-130	VPKAS, Almora
11	HAFB-3	HARP, Ranchi	28	Aparna	Prabhakar Hybrid Seed Company, Hyderabad
12	HAFB-4	HARP, Ranchi	29	Chandini	Sutton and Sons (India) Pvt. Ltd.
13	IVRFB-1	IIVR, Varanasi	30	Falguni	Seminis Vegetable Seeds (India) Ltd.
14	IVFB-1	IIVR, Varanasi	31	Surya	Solar Seeds
15	IVFB-2	IIVR, Varanasi	32	Arka Komal	IIHR, Hasarghata
16	IVFB-3	IIVR, Varanasi	33	Contender	CSKHPKV, Palampur
17	JFB-97-1	GAU, Junagadh			

Table 2. Pooled analysis of variance over two years for different characters in French bean

Traits	Mean Squares				
	Source	Genotypes	Environment	Genotype × Environment Interactions	Pooled error
	df	32	1	32	128
Days to 50 % flowering		16.65*	8.91*	6.91*	1.81*
Days to first marketable picking		15.83*	2.44*	6.47*	2.85*
Average pod weight (g)		3.28*	213.72*	1.62*	0.38
Branches/plant		0.99	0.21	0.99	0.15
Pods /plant		112.21*	0.703	38.31*	3.45*
Fresh pod yield/plant (g)		3806.07*	50345.38*	1330.8*	25.45*
Days to seed maturity		15.69*	861.5*	9.33*	1.03
Pod length (cm)		16.89*	0.39	1.40	0.72
Seeds / pod		1.30	0.633	0.23	0.14
Biological yield/plant (g)		362.23*	5083.97*	203.55*	5.049*
Plant height (cm)		40.12*	145.19*	16.30*	4.94*
100-seed weight(g)		389.31*	57.89*	35.26*	7.04*
Seed yield/plant (g)		399.87*	477.71*	126.65*	17.47*

*Significant at P≤0.05

Table 3. Estimates of coefficient of variations and other genetic parameters for different seed traits in French bean pooled over two years

Traits	Range	Mean	Coefficients of variation			Heritab-ility (h^2) (%)	Genetic advance	Genetic advance (%) of mean
			Phenot-ypic (PCV)	Genotypic (GCV)	Environmental (ECV)			
Days to 50 % flowering	40.00-46.67	42.42	4.88	3.71	3.17	57.74	2.46	5.81
Average pod weight (g)	4.52-8.23	5.96	15.63	11.66	10.40	55.69	1.07	17.93
Branches /plant	2.68-4.27	3.29	17.60	13.12	11.73	55.57	0.66	20.15
Pods /plant	9.13-26.7	14.96	31.05	28.46	12.41	84.01	8.04	57.72
Fresh pod yield/plant (g)	43.65-141.5	87.93	29.15	28.58	5.74	96.12	57.72	53.74
Days to seed maturity	78.50-84.5	81.44	2.29	1.92	1.24	70.40	2.70	3.32
Pod length (cm)	10.55-16.10	13.82	13.38	11.89	6.14	78.91	3.00	21.75
Seeds/ pod	4.03-6.33	5.59	10.33	7.87	6.69	58.00	0.69	12.34
Biological yield/plant (g)	14.95-50.67	29.23	27.49	26.40	7.69	92.18	15.26	52.21
Plant height (cm)	29.37-39.57	35.47	9.27	6.83	6.27	54.28	3.68	10.36
100 -seed weight (g)	14.88-48.95	33.00	25.48	24.18	8.04	90.06	15.60	47.27
Seed yield/plant (g)	12.58-38.57	22.26	40.50	35.88	18.78	78.49	14.57	65.48

traits was observed for fresh pod yield/plant, pods/plant, 100-seed weight, days to first marketable picking and days to 50% flowering. High estimates of PCV and GCV for seed yield/plant indicates that selection and breeding initiatives can be taken up immediately (Ordonez *et al.* 2005). On the other hand, fresh pod yield/plant, pods/plant, biological yield/plant, 100-seed weight, branches/plant, pod length and average pod weight had moderate estimates of PCV and GCV suggesting that these traits should be considered cautiously for direct selection. Singh *et al.* (2007) and Raffi and Nath (2004) also supported this theory for pod length. High PCV with moderate GCV was recorded for pods /plant.

High heritability estimates were observed for fresh pod yield/plant, biological yield/plant, 100-seed weight, pods/plant, pod length, seed yield/plant and days to seed maturity (Table 3). This reveals the presence of additive gene action and the characters can be fixed by resorting to selection. High heritability estimates were also recorded by Mishra *et al.* (2008) for 100-seed weight and fresh pod yield/plant, Junaif *et al.* (2010) for number of pods/plant and Salgotra and Gupta (2005) for 100-seed weight and seed yield/plant in their respective studies with different french bean germplasm under variable environments. Johnson *et al.* (1955) stressed that for estimating the actual effects of selection, heritability alone

cannot be the sole indicator for improvement since high heritability does not mean high expected genetic advance. Hence, prediction on the basis of both heritability and genetic advance simultaneously could be more useful. High heritability along with high genetic advance was recorded for seed yield/plant, fresh pod yield/plant, biological yield/plant and 100-seed weight (Table 3). This suggested the presence of additive gene action and hence, these characters are likely to respond better to selection. The manifestation of additive gene action for pods/plant and seed yield/plant was also advocated by Singh *et al*. (2007) and Walling and Chaturvedi (2014).

Correlation studies revealed that seed yield/plant was positively and significantly correlated with fresh pod yield/plant, pods/plant, 100-seed weight, average pod weight, plant height, pod length, biological yield/plant and branches/plant (Table 4). From different sets of genetic material and environmental conditions, Karasu and Oz (2010) and Bezaweletaw *et al*. (2006) also observed positive association of seed yield with some of these traits. In contrary, Shukla *et al*. (2006) observed a negative correlation between plant height and seed yield. On the other hand, seed yield/plant was negatively associated with days to seed maturity which was also observed by Sofi *et al*. (2011). This association signifies that early maturing genotypes tend to have high pod yield which is quite relevant under those conditions where seed maturity coincided with early onset of monsoons and thereby affected the total seed yield.

Table 4. Estimates of phenotypic (P) and genotypic (G) correlation coefficients for different pairs of horticultural traits in French bean pooled over two years

Traits		Average pod weight (g)	Branches/ plant	Pods/ plant	Fresh pod yield/ plant (g)	Days to seed maturity	Pod length (cm)	Seeds/ pod	Biological yield/ plant (g)	Plant height (cm)	100-seed weight (g)	Seed yield/ plant (g)
Days to 50 % flowering	P	0.086	0.238*	0.146	0.229*	0.254*	-0.045	-0.002	0.347*	0.220*	-0.325*	-0.111
	G	0.307*	0.360*	0.277*	0.370*	0.462*	-0.05	0.084	0.394*	0.409*	-0.475*	-0.068
Average pod weight (g)	P		-0.009	-0.183	0.290*	-0.006	0.326*	-0.071	0.156	0.032	0.301*	0.094
	G		-0.262*	-0.065	0.332*	-0.019	0.610*	-0.179	0.325*	0.231*	0.449*	0.474*
Branches/plant	P			0.423*	0.397*	0.276*	-0.412*	0.18	0.393*	0.268*	-0.298*	0.119
	G			0.697*	0.595*	0.446*	-0.602*	0.315*	0.558*	0.509*	-0.440*	0.203*
Pods/plant	P				0.851*	0.253*	-0.180	0.202*	0.278*	0.335*	-0.183	0.633*
	G				0.915*	0.381*	-0.335*	0.343*	0.349*	0.417*	-0.268*	0.582*
Fresh pod yield/ plant (g)	P					0.246*	-0.002	0.210*	0.389*	0.344*	-0.020	0.672*
	G					0.272*	-0.077	0.316*	0.472*	0.506*	-0.065	0.763*
Days to seed maturity	P						-0.313*	-0.130	0.203*	0.201*	-0.350*	-0.174
	G						-0.583*	0.347*	0.245*	0.422*	-0.603*	-0.248*
Pod length (cm)	P								-0.058	0.007	0.539*	0.283*
	G								-0.059	-0.008	0.660*	0.356*
Seeds/ pod	P								0.151	0.192	-0.323*	0.144
	G								0.236*	0.260*	-0.449*	0.103
Biological yield/ plant (g)	P									0.261*	-0.202*	0.127
	G									0.414*	-0.187	0.223*
Plant height (cm)	P										-0.086	0.273*
	G										-0.007	0.386*
100-seed weight (g)	P											0.474*
	G											0.527*

*Significant at P≤0.05

Pods/plant, branches/plant, fresh pod yield/plant, days to seed maturity, biological yield/plant, seeds/ pod and plant height showed a positive and significant association with each other. This reflects that the performance of these traits is inter-dependable and a due consideration must be paid towards these traits for improvement in French bean. Angadi et al. (2012) also observed positive association among these traits. A significant and positive correlation between seed yield/ plant and fresh pod yield is of significance as it indicated the possibilities of development of dual purpose French bean variety(ies) which would be suitable for both vegetable and dry bean as per suitability and preference of consumers.

The path analysis study revealed that the direct effects obtained at genotypic level were markedly different from those at phenotypic level (Table 5) which might be due to varying degree of influence of environment on various traits studied. This fact was also revealed from the results of component variance analysis and correlation at the environmental level. In few cases, like the direct effect of average pod weight, number of branches/plant, fresh pod yield/plant and biological yield/plant on seed yield/ plant were observed to be of opposite sign (positive to negative and vice-versa) at corresponding phenotypic and genotypic levels. Such a change in direction and magnitude of direct and indirect effects might be due to the environmental factors influencing various traits under study.

Pods/plant and average pod weight exhibited maximum positive direct effect at genotypic level followed by 100-seed weight, seeds/ pod, number of branches/plant and plant height on seed yield/plant. Salehi *et al.* (2010) and Roy *et al.* (2006) also observed direct contribution of these traits on seed yield. Further, it was observed that number of pods/plant had the maximum indirect contribution on the total association of number of branches/plant, fresh pod yield/plant, biological yield/ plant and plant height at both phenotypic and genotypic levels, which resulted into positive and significant association of these traits with seed yield.

The low magnitude of residual effects at phenotypic and genotypic levels indicated that the traits included in the present investigation accounted for most of the variation present in the dependent variable. In view of the direct and indirect contributions of component traits, selection on the basis of pods/plant, average pod weight, 100-seed weight, seeds/pod and branches/plant for seed yield/plant would be a paying proposition for evolving high yielding genotypes.

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Table 5. Direct and indirect effects of yield traits on seed yield in French bean in pooled over two years

Trait		Days to 50 % flowering	Average pod weight (g)	Branches/ plant	Pods/plant	Fresh pod yield/plant (g)	Days to seed maturity	Pod length (cm)	Seeds/ pod	Biological yield/plant (g)	Plant height (cm)	100-seed weight (g)
Days to 50 % flowering	P	**-0.032**	-0.003	-0.006	-0.005	-0.007	-0.008	0.001	0.000	-0.011	-0.007	0.010
	G	**-0.071**	-0.022	-0.025	-0.020	-0.026	-0.033	0.004	-0.006	-0.028	-0.029	0.034
Average pod weight (g)	P	-0.009	**-0.099**	0.009	0.018	-0.029	0.001	-0.032	0.007	-0.015	-0.003	-0.029
	G	0.201	**0.654**	-0.171	-0.043	0.217	-0.126	0.399	-0.117	0.213	0.151	0.294
Branches /plant	P	-0.005	0.002	**-0.021**	-0.009	-0.008	-0.006	0.008	-0.004	-0.008	-0.006	0.006
	G	0.075	-0.054	**0.209**	0.146	0.125	0.093	-0.126	0.066	0.117	0.107	-0.092
Pods/plant	P	0.064	-0.080	0.185	**0.438**	0.372	0.111	-0.079	0.088	0.122	0.147	-0.080
	G	0.374	-0.088	0.940	**1.348**	1.233	0.513	-0.452	0.462	0.470	0.563	-0.361
Fresh pod yield/plant (g)	P	0.076	0.096	0.132	0.283	**0.333**	0.082	-0.001	0.070	0.130	0.115	-0.007
	G	-0.279	-0.251	-0.450	-0.692	**-0.756**	-0.279	0.058	-0.239	-0.357	-0.383	0.168
Days to seed maturity	P	-0.047	0.001	-0.051	-0.047	-0.046	**-0.185**	0.058	-0.023	-0.038	-0.037	0.065
	G	-0.129	0.054	-0.124	-0.106	-0.076	**-0.012**	0.163	-0.105	-0.068	-0.118	0.049
Pod length (cm)	P	-0.003	0.018	-0.023	-0.010	0.000	-0.018	**0.056**	-0.007	-0.003	0.000	0.030
	G	-0.001	0.012	-0.012	-0.001	-0.002	0.109	**0.020**	-0.005	-0.001	0.000	0.013
Seeds / pod	P	0.000	-0.012	0.031	0.035	0.036	0.022	-0.023	**0.174**	0.026	0.033	-0.056
	G	0.024	-0.052	0.091	0.099	0.091	-0.034	-0.069	**0.289**	0.068	0.075	-0.130
Biological yield/plant (g)	P	0.001	0.003	0.006	0.004	0.006	0.003	-0.001	0.002	**0.016**	0.004	-0.003
	G	-0.054	-0.045	-0.077	-0.048	-0.065	0.048	0.008	-0.033	**-0.138**	-0.058	0.026
Plant height (cm)	P	0.016	0.002	0.020	0.025	0.025	0.015	0.001	0.014	0.019	0.073	-0.006
	G	0.046	0.026	0.058	0.047	0.057	0.322	-0.001	0.029	0.047	**0.113**	-0.007
100-seed weight(g)	P	-0.177	0.164	-0.162	-0.100	-0.010	-0.191	0.294	-0.176	-0.110	-0.047	**0.545**
	G	-0.254	0.240	-0.235	-0.143	-0.035	-0.206	0.353	-0.240	-0.100	-0.036	**0.534**
Correlation	P	-0.111	0.094	0.119	0.633*	0.672*	-0.174	0.283*	0.144	0.127	0.273*	0.474*
	G	-0.068	0.474*	0.203*	0.582*	0.763*	-0.248*	0.356*	0.103	0.223*	0.386*	0.527*

Significance at P≤0.05; Residual effect at phenotypic level=0.249 and at genotypic level=0.19; bold digits corresponds to direct effects

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Specific Characteristics Features of Farmers' Varieties of Rice (*Oryza sativa* L.) for Testing of Distinctiveness

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Distinctiveness of a genotype is compulsory for registration under PPV & FR Act (2001). Some of the special characters of rice have been already included in the *Table of Characteristics* in the "Guidelines for Conduct of Test for Distinctiveness, Uniformity and Stability (DUS) on rice (*Oryza sativa* L.)" published by PPV & FR Authority, Government of India for rice, however some other characters which may be included in the descriptor and/or some existing trait in the descriptor may be modified. Six farmers' varieties with some special characteristics were studied for their panicle and grain characters including their special characters. Significant variability was observed for all the panicle and grain characters. In this study, we identified multiple kernel character (reference variety- *Jugal*) which may be included in the descriptors for rice as a novel trait. Occurrence of single, double and triple kernels per spikelet was 53.9, 42.2 and 3.9%, respectively. Only the colour of the sterile lemma has been included in the existing descriptor, whereas the length of the sterile lemma may also be included. The average length of the sterile lemma in *Rami Gelee* was 9.09 mm, whereas the length of fertile lemma was 8.67 mm. Apart from these two characters, in this communication we have described three more characters, namely clustered panicle, coloured kernel and dark purple coloured rice plant.

Key Words: Black kernel, Clustered panicle, Famers' varieties, Long sterile lemma, Multiple kernel, Purple plant, Rice

Introduction

Rice is one of the oldest crop domesticated and during the long period of evolution and domestication, a wide array of crop variability generated by natural and artificial selection in rice. Rice breeders have been exploiting this potential variation in many different and creative ways in crop improvement programmes. The first opportunity to take advantage of the local landraces and wild relatives' with unique characteristics was through searching of the existence of genes for important characters. As Assam and adjoining states are the part of primary centre (The Hindustan Centre of Origin, which includes Myanmar, Assam, Malaya Archipelago, Java, Borneo, Sumatra and Philippinese) of origin of rice the possibility of existence of farmer's varieties (FVs) with unique character(s) may be high. India is home to wide varieties of rice cultivars, landraces and many lesser known types that have been under cultivation since ages by indigenous farmers as-well-as local entrepreneurs (Vinita *et al*., 2013). India is the home for more than 75,000 local cultivars/landraces of rice (Paroda and Malik, 1990; Khush, 1997). Studies have revealed that these FVs possess useful genes for rice crop improvement programme (Roy, 2013, Roy *et al*., 2013).

West Bengal is called as 'bowl of rice' with over 450 rice landraces (Deb, 2005; Chatterjee *et al*., 2008). Rice is cultivated here on over 65% area under agricultural crops (Adhikari *et al*., 2012) in three different seasons *viz*., *Aus* (autumn rice), *Aman* (winter rice) and *Boro* (summer rice). The ecotypes of rice, spontaneously evolved in the state, are so diverse and different that scientists at one time coined them as *Oryza sativa* var. *benghalensis* (Chatterjee *et al*., 2008). The unique diversity in landraces/local cultivars of rice from West Bengal is well recognized for significant traits like aroma, taste and disease resistance. However, there are some special characters which may or may not have much importance in respect of consumers' preference, but have immense importance for rice breeder as distinctive character during registration of variety under PPV&FR Act (2001). Considering the immense variability in FVs, some special characters of six FVs in the rice repository of Uttar Banga Krishi Viswavidyalaya have been studied.

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Materials and Methods

The experimental materials consisted of six FVs of rice namely, Rami Gelee, Thuri, Sadabhot Kalo, Kalakali, Kharadhan and Jugal (Table 1). The crop was grown during *Kharif* seasons 2013 and 2014 in randomized block design with four replications. The collected FVs were grown in the University Research Farm, Pundibari, situated at 26°19′ N latitude, 99°23′ E longitude and at a height of 43 MSL. Twenty eight days seedlings were transplanted in the main field. The spacing was maintained at 30 × 25 cm. The standard cultural practices were followed during the crop season to obtain good crop stand in the main field. Each plots consisted of fifteen rows of 5 m length. Five plants from inner rows were randomly selected for taking observation on various quantitative and qualitative characters. Observations were taken on special characters namely, colour and length of sterile lemma of Rami Gelee, clustering pattern of Thuri, presence of double and triple kernels of Jugal, purple coloured leaf and leaf sheath of Khara dhan and kernel colour of Sadabhat Kalo. Ten spikelets of Rami Gelee were taken to measure the length of sterile lemma and the average values were recorded. Ten panicles were taken to record the clustering pattern of Thuri. Digital Varner's caliper was used to measure the length of the sterile lemma and grain dimensions of all the varieties. The average values of the data were reported for various quantitative characters. For the assessment of all colour characteristics, the latest Royal Horticultural Society (RHS) colour chart was used. In addition of those special characters, grain characteristics of above mentioned varieties also have been studied. The data were subjected to standard statistical methods of analysis of variance (ANOVA) using AgRes Statistical Software, (c) 1994 Pascal Intl Software Solutions, Version 3.01 and significant differences were compared by LSD.

Results and Discussion

Analysis of variance showed highly significant differences among the FVs for all the characters studied (Table 2), namely panicle length, number of filled grains per panicle, number chaffy grains per panicle, grain density, spikelet sterility, length of sterile lemma, grain length, grain width, grain L:B ratio and 100-grains weight.

Table 1. Special features of six Farmers' Varieties of rice of West Bengal

S. No.	Genotype	Sources	Special features
1.	Thuri	Tarai Research Society, Alipurduar, W.B.	Clustered panicle
2.	Jugal	Sat Mile Satish Club, Cooch Behar, W.B.	About 60% of the spikelets bears doubled kernel
3.	Rami Gelee	Sat Mile Satish Club, Cooch Behar, W.B.	Sterile lemmas are white in colour and longer than the fertile lemma and palea
4.	Sadabhotkalo,	Tarai Research Society, Alipurduar, W.B.	Lemma and palea of are straw coloured, however, dehusked rice is dark purple to black
5.	Kalakali	Tarai Research Society, Alipurduar, W.B.	Lemma and palea of are purple spot/furrows on straw, however, dehusked rice is light brown
6.	Khara dhan	Tarai Research Society, Alipurduar, W.B.	The colour of fully opened leaves and leaf sheath is deep purple, resistant to lodging

Table 2. ANOVA for the effect of plant growth regulators on callus induction, plantlet regeneration and plantlets per responding callus

Genotypes/Characters	Sources of variation			
	Total (23)	Replication (3)	Treatment (5)	Error (15)
Panicle Length	7.240	0.657	30.768**	0.713
Number of filled grains per panicle	1600.992	156.561	7060.976**	69.884
Number chaffy grains per panicle	215.799	20.664	910.289**	23.329
Grain density	5.702	0.419	24.998**	0.327
Spikelet sterility	19.327	1.424	83.933**	1.373
Length of sterile lemma	6.142	0.011	28.238**	0.003
Grain length	0.778	0.010	3.547**	0.008
Grain width	0.141	0.008	0.633**	0.004
Grain L:B ratio	0.118	0.001	0.539**	0.001
100-grain weight	0.755	0.001	3.465**	0.003

** denote significance at P= 0.01

Clustered Panicle

In general, a pedicel bears one spikelet in a panicle. In the repository of FVs of Uttar Banga Krishi Viswavidyalaya, *Narkeldari* and *Thuri* showed clustered panicle (Figs. 2A-B). The frequency of distribution of spikelet per clusters in Thuri varied from 1 to 4 and from 1 to 6 Narkeldari (Table 3). Clustered habit appears to result mainly from reduction in pedicel length. This feature resulted high grain density in a panicle (Table 4). This result also corroborated with the findings of Chakrabarty *et al*. (2012), for the FV *Khejurchari*. Findings of Sahu *et al*. (2014) for the FVs Amajhopa, Kaudidhul, Chhindguchhi, Nariyal Phool and Amaruthi also confirmed the availability of clustered panicles in the FVs. Panicle and grain characteristic of Thuri have been given in Table 4. It showed highest grain density and reduced spikelet sterility. Its grain type is long bold, straw coloured lemma and palea, and light brown kernel colour.

Table 3. Frequency distribution of spikelet clusters in Thuri and Narkeldari with cluster spikelet

No. of spikelets occurred in cluster	Frequency of spikelet-clusters*	
	Thuri	Narkeldari
1	59.0	10.2
2	20.4	15.3
3	42.0	60.5
4	15.6	11.8
5	–	1.2
6	–	1.0

*Percentage; average of five panicles

Multiple Kernels Per Spikelet

The cultivar *Jugal* had multiple (2-3) kernels per spikelet (Figs. 1C-D). Occurrence of single, double and triple kernels per spikelet in *Jugal* was 53.9, 42.2 and 3.9%, respectively. This was also studied by Chakrabarty *et al*. (2012) and they found that the occurrence of single, double and triple kernels per spikelet were 53.7, 41.3 and 5.0 per cent, respectively. This result corroborated by the findings of Jarvis *et al*. (2008) and Kumar *et al*. (2010). Paul *et al*. (2012) also observed twin and triplet seedlings in up to 20 % of the seeds. The multiple embryos, mostly twins and triplets, and rarely quadruplets, developed through sequential cleavage from a single zygotic embryo in each ovule (Paul *et al*. 2012). As per the observation of Paul *et al*. (2012), multiple seedlings are due to sequential proliferation and cleavage of the zygotic embryos, the nucellar tissue was not involved in multiple embryo development. Grain characteristic of *Jugal* been given in Table 4. Jugal had medium bold grain, straw coloured lemma and palea, and brown kernel.

Rice is single seeded fruit and generally it bears one kernel per spikelet. Old literature on developmental anatomy concludes that rice spikelets are primitively three-grained, of which the two lateral have become vestigial (hence "sterile lemma"). It would be interesting to know if the multi-grained spikelets are a reversion to primitive type, or a new splitting of the central grain (Annonymous, 2015).

Table 4. Grain panicle characters of six farmers' varieties of rice of West Bengal

Characters/ Genotype	Thuri	Jugal	Rami Gelee	Sadabhatkalo	Kalakali	Khara dhan
Panicle length (cm)	21.76 e	24.53 d	28.22 b	24.90 cd	25.95 c	29.52 a
No. of filled grains/panicle	282.10 a	178.78 cd	169.32 d	187.80 c	179.40 cd	211.60 b
No. of chaffy grains/panicle	48.75 c	38.45 b	29.16 a	63.60 d	37.80 b	66.60 d
Grain density	12.96 a	7.25 b	6.00 c	7.54 b	6.91 b	7.17 b
Spikelet sterility (%)	14.73 a	17.70 b	14.69 a	25.29 c	17.40 b	23.93 c
Length of sterile lemma	2.54 e	2.64 d	**9.09 a**	2.79 c	2.92 b	2.17 f
Grain length (mm)	8.10 d	8.26 c	8.67 b	9.86 a	7.46 e	7.24 f
Grain breadth (mm)	2.65 c	3.12 b	3.45 a	3.14 b	2.36 d	3.16 b
L:B ratio	3.05 b	2.65 c	2.51 d	3.14 a	3.16 a	2.29 e
100-grains weight (g)	2.43 d	2.38 d	3.11 b	2.58 c	1.62 e	4.38 a
Grain type	LB	MB	LB	LB	LB	MB
Lemma and palea colour	S	S	DB	S	PS/FS	PS/FS
Kernel colour	LB	B	LB	DP	LB	P

*values bearing same letter in the row are not significantly different at P = 0.05 of LSD; LB: Long bold; MB: Medium bold; S: Straw; B: Brown; DB: Dark brown; LB: Light brown; PS: Purple spot; FS: Furrows on straw; P: Purple; DP: Dark purple

This exceptional character which is not present in the *Table of Characteristics* in the "Guidelines for Conduct of Test for Distinctiveness, Uniformity and Stability on rice (*Oryza sativa* L.)" published by PPV & FR Authority, Government of India for rice (PPV&FRA, 2007). This may be included as distinct character for varietal identification and registration under PPV & FR Act (2001).

Long Sterile Lemma

Generally the sterile lemmas are much smaller in size than the fertile lemma, and they do not bear flower, hence their name "sterile". The sterile lemmas of *Rami Gelee* most of the time exceeded fertile lemma and palea by length (Figs. 1E-F). The average length of the sterile lemma was 9.09 mm, whereas the length of fertile lemma was 8.67 mm (Table 4). *Rami Gelee* exhibited longest sterile lemma (Table 4). The sterile lemma of rest all the FVs were much shorter than the fertile lemma. The colour of the fertile lemma is dark brown, while the sterile lemmas are simple white (Fig. 1E-F). Other panicle and grain characteristics have been given in (Table 4). Grain type of *Rami Gelee* was long bold, lemma and palea colour was dark brown, and colour of the kernel was light brown.

As per description of Chang *et al*. (1965), a spikelet consists of a minute axis (rachilla) on which a single floret is borne in the axils of 2-ranked bracts. The bracts of the lower pair on the rachilla, being always sterile, are the 'sterile lemmas'. The upper bracts or the flowering glumes consist of the lemma (fertile lemma) and palea. The lemma, palea, and the included flower form the floret. The lemma and palea were seldom exceeding one-third the length of the latter (Chang *et al*., 1965). The sterile lemmas may be equal or unequal in size, the upper one generally being larger. The colour of sterile lemma has been included in the *Table of Characteristics* in the "Guidelines for Conduct of Test for Distinctiveness, Uniformity and Stability on rice (*Oryza sativa* L.)" published by PPVFR Authority, Government of India (PPV&FRA, 2007). However, white coloured sterile lemma has not also been included in the guidelines. In the guidelines only four colour have been mentioned, namely, straw, gold, red and purple. The sterile colour of *Rami Gelee* is white in colour (Fig. 1F). The length of sterile lemma also has not been included in the guidelines. So, these two special characters may be included as 'distinct character' for varietal identification and subsequently registration under PPV & FR Act (2001).

Dark Purpled Kernel

Lemma and pelea of *Sadabhot kalo* (Fig. G) and *Kalakali* were straw coloured, however, dehusked rice were dark purple to black (Fig. H) and cooked rice also black. It has a dark purple to black bran layer (inner protective layers on a rice grain), but it is notable that this colour continues through to grain itself so when the kernels are milled, they retained the purple to lavender colour, depending upon the degree of milling. Other panicle and grain characteristics have been given in the Table 4. Grain type was long bold, lemma and palea colour was purple spot or furrows on straw, and colour of the kernel was light brown.

The most common rice consumed by humans is white rice, followed by brown rice. However, rice genotypes with either red/purple or black bran layer have been cultivated for a long time in Asia (Ahuja *et al*., 2007). Coloured rice is reported to be potent sources of antioxidants and their consumption is encouraged (Yawadio *et al*., 2007; Anggraini *et al*., 2015). Black rice contains relatively high anthocyanin (primarily *cyaniding-3-O-glucoside and peonidin 3-O-glucoside*) in the pericarp layer which gives the dark purple color (Ryu *et al*., 1998; Takashi *et al*., 2001; Kristamtini *et al*., 2012). Anthocyanin is known for their bioactive properties and recognized as health-enhancing substances due to their antioxidant activities, anti-inflammatory, anticancer, anti-atherogenic, and anti-hypoglycemic effects (Wang and Stoner, 2008). Black rice is low in sugar but packed with healthy fibre and plant compounds that combat heart disease and cancer, according to scientists (Sutharut and Sudarat, 2012). When rice is processed, millers remove the outer layers of the grains to produce brown rice or more refined white rice, the kind most widely consumed in the West. Brown rice is said to be more nutritious because it has higher levels of healthy vitamin E compounds and antioxidants. But varieties of rice that are black or purple in colour are healthier still. Some scientist claimed that black rice is the new cancer-fighting rice (http://www.dailymail.co.uk/health/article-1306356/Black-rice-new-cancer-fighting-superfood-claim-scientists.html#ixzz3YKu9R4lg).

Research suggests that the dark plant antioxidants, which mop up harmful molecules, can help protect arteries and prevent the DNA damage that leads to cancer. Colored rice have important roles in reducing risk of cancer and other chronic diseases because of their free radicals scavenging capacities (Wang and Stoner,

2008; Shih *et al*., 2007; Elisia *et al*., 2007; Elisia and Kitts, 2008). Black rice also contains higher levels of proteins, vitamins and minerals than common white rice (Suzuki *et al.*, 2004).

Purpled Leaf and Leaf Sheath

The colour of fully opened leaves and leaf sheath of Khara dhan are deep purple (Fig. 1 I). Detail of its importance in photosynthesis and other activities yet to be studied. Sakamoto *et al*. (2001) studied about the purple locus of rice and they stated that the purple leaf (*Pl*) locus of rice affects regulation of anthocyanin biosynthesis in various plant tissues. It is long duration, photoperiod-sensitive cultivar. This FV is almost Registrant to major insect pests and disease pathogens prevailing in northern part of West Bengal. It was also found to be resistant to lodging. So, this FV may be used in resistant breeding as a donor against lodging, insect pests and disease pathogens. Other panicle and grain characteristics have been given in the Table 4. It possessed highest 100-grain weigh (4.38 g). Grain type was medium bold, lemma

Fig. 1. Pictorial depiction of special characters of Farmers' Varieties of rice. A) Undehusked grain of Thuri, B) Panicle of Thuri, C) Undehusked grain of Jugal, occurrence of single, double and triple kernels per spikelet in the FV *Jugal* was 53.9, 42.2 and 3.9%, respectively, D) Dehusked grain of Jugal (one kerneled grain and two kerneled grain, respectively), E) Unhusked grain of Rami Galee F). White coloured lemmas of Rami Gelee, which is generally longer than the fertile lemma and palea, G) Unhusked grain of Sadabhat Kalo, H) Dehusked grain of Sadabhat Kalo, I) Dark purpled leaf and leaf sheath of Khara dhan.

and palea colour was purple spot or furrows on straw, and colour of the kernel was purple.

Nowadays, the common people are health conscious. As per the recent finding, brown rice is said to be more nutritious than white rice (polished rice) because it has higher levels of healthy vitamin E compounds and antioxidants, but, varieties of rice that are black or purple in colour are healthier still. Thus the *Kalakali* and *Sadabhot kalo* may be popularized to improve the nutritional quality of meals of Asian and African people in particular and Latin American in general. The multiple kernel and sterile lemma colour as well as length of sterile lemma may be included in the *Table of Characteristics* in the "Guidelines for Conduct of Test for Distinctiveness, Uniformity and Stability on Rice (*Oryza sativa* L.)" published by PPVFR Authority, Government of India for Rice (PPV&FRA, 2007) for identification and subsequently registration of variety under PPV & FR Act (2001). The colour of the fertile lemma has been included in the guidelines; however the length of sterile lemma has not been included in the guidelines. So, this special character may be included as distinct character for varietal identification and subsequently registration under PPV & FR Act (2001).

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Screening for Moisture Stress Tolerant Genotypes in *C. olitorius* L. on the Basis of Seedling Characters

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Most cultivars of *C. olitorius* (tossa jute) suffer extremely during early growth phase either due to lean season or insufficient rainfall to maintain steady growth. Tolerant genotypes may overcome this problem maintaining sustainable growth at early growth. Sixty genotypes of *C. olitorius* were evaluated for identification of moisture stress tolerant genotypes using PEG, in field and pot under rainfed condition. The characters considered were root length, root volume, shoot length, root fresh weight, shoot fresh weight, leaf fresh weight, root dry weight, shoot dry weight, leaf dry weight, and tolerance index which showed significant differences among genotypes under different water regimes for stress tolerance. On the basis of seedling characters OEX 29 was found to be consistent by highly tolerant to moisture stress after evaluation in laboratory, field and pot culture. Genotypes OIJ 177 and OIN 791 showed tolerance, whereas, JRO 524 followed by JRO 632, JRO3690, JRO 8432, OIJ214 and OIN 970 were found to be the most susceptible genotypes.

Key Words: *Corchorus olitorius* L., Drought tolerant, Moisture stress, PEG 6000, Seedling characters

Introduction

Drought is a multifaceted stress condition with respect to timing and severity, ranging from long drought seasons where rainfall is much lower than demand, to short periods where plants depend completely on available soil water which severely impairs plant growth and development, performances and productivity (Lafitte *et al.,* 2006). However, the susceptibility of plant to drought stress varies among plant species and their developmental stages (Demirevska *et al.,* 2009). Two cultivated jute species *viz.*, *Corchorus capsularis* and *Corchorus olitorius*, are grown for fibre in South East Asian countries like India, Bangladesh, Nepal, China, Indonesia, Thailand, Myanmar and some South American countries. West Bengal in India, the main jute producing state of which 80 per cent of its total cultivated area (6.4 lakhs ha) is at the mercy of rain particularly at the early growth stage (Basu, 1997). Therefore, timely sowing and assured rainfall play important role for successful cultivation but farmers are not always in a position to afford improved technology with artificial irrigation. Owing to the erratic nature of rainfall the crop is often subjected to phasic spell of moisture stress during early growth stage. The germination of delicate jute seed is often hampered due to an un-assured moisture reserve in soil coupled with high temperature in summer months (March to May), which frequently leads to crop failure or poor fibre yield that necessitates development of tolerant genotypes which can survive moisture stress with sustainable growth at initial period. It has been noticed that if jute crop can survive the adverse initial dry spell then with the advent of monsoon with favourable weather conditions like adequate rainfall, high relative humidity and warm temperature it will show luxuriant growth with high fibre yield. Therefore, to tackle the moisture stress at the early growth stage identification or development of moisture stress tolerant genotypes could be one of the best options to accomplish good harvest from jute, particularly from more profitable *olitorius* species. The present investigation was initiated for identification of such tolerant genotypes by carrying out different experiments in laboratory, field and pot for evaluation of sixty genotypes. Artificial stress was created with PEG 6000 solution in laboratory and experiments in field and pot culture were conducted under rainfed condition.

Materials and Methods

The experimental materials consisted of sixty genotypes of *Corchorus olitorius* collected from All India Network Project on Jute and Allied Fibres, Kalyani

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Centre, B.C.K.V. in collaboration with CRIJAF, ICAR, Barrackpore, Kolkata. Out of sixty genotypes, 25 were indigenous, 16 standard varieties, 14 accessions of International Jute Organization (IJO) and five exotic varieties. The identified genotypes were evaluated to identify moisture stress tolerant genotypes by conducting experiments in laboratory using PEG 6000, field and earthen pots under rainfed condition for consecutive two years (2012 and 2013). In each experiment percentage of reduction was calculated by, % R = [1–Mean performance as measured for a character under treatment or rainfed condition / Mean performance as measured for the same character under control or irrigated condition] x 100.

Laboratory Experiment

The laboratory experiment was arranged in factorial completely randomized design (FCRD) with three replications and with two factors namely genotypes and irrigation levels i.e. control and stress. In the beginning to find out the suitable external water potential, all the sixty genotypes were tested in three different ranges of external water potential (viz, -2.0, -3.0 and -4.0 bars). These solutions were prepared using PEG 6000 following the method described by Michael and Kaufmann (1973). On the basis of growth performance of the seedlings of different genotypes grown in all three ranges of external water potential of PEG, a suitable range at -3.0 bar was identified. Subsequently all the genotypes were evaluated in -3.0 bar of PEG 6000 solution by growing them in glass plate and data were recorded for the seedling characters viz. root length (cm), shoot length (cm), root fresh weight (g), shoot fresh weight (g), leaf fresh weight (g), root dry weight (g), shoot dry weight (g) and leaf dry weight (g). From the above data tolerance index (TI) was calculated as per Garg and Singla (2004). For identification of stress tolerant genotypes tolerance index could be an important selection indicator and high value of tolerance index implied tolerance of the genotypes under stress. The tolerance index was calculated as = TI = [Dry weight of seedling grown in water stress condition / Dry weight of seedling of the same genotype grown in non stress condition (Control condition)] X 100.

Field Experiment

These genotypes were grown under field in two varying water regimes viz, i) rainfed condition and ii) irrigation conditions. The experiment in each environment was laid out in Randomized Block Design with three replications. In each replication each genotype was grown in a plot of five rows of three meter length maintaining 30 cm space between the rows. The size of each plot was 3 m X 1.5 m and plot to plot distance was 0.5 m. Sowing was done in two consecutive years, on 5th April 2012 and 29th March 2013. Recommended doses of major nutrients (N, P and K) were applied and normal cultural practices were followed. Randomly ten 21 days old seedling from both regimes, the moisture stress and the irrigated, were selected from each replication. The observations on each genotypes were recorded for nine characters viz. root length (cm), root volume (cc), root fresh weight (g), root dry weight (g), shoot length (cm), shoot fresh weight (g), shoot dry weight (g), leaf fresh weight (g), leaf dry weight (g) and tolerance index (%).

Pot Experiment

Earthen pots of 25 cm and 30 cm base and top diameter, respectively and 35 cm height, were filled with FYM mixed soil and fertilizers recommended as basal dose. In each pot thirty seeds were sown in two consecutive years, on 11th April 2012 and 10th April 2013 and the seedlings were thinned out every week. Seven seedlings were maintained in each pot. Five seedlings from each pot were uprooted carefully on 21 days after sowing (DAS) and similar observations were recorded on different characters as mentioned for field experiment. Correlations among characters were calculated in all three experiments. All the statistical analysis carried out with the help of IndoStat software version 8.6. (Indostat services, Hyderabad).

Results and Discussion

Laboratory Experiment

Analysis of variance from laboratory experiment showed significant treatment and genotype x treatment interactions for all the characters which revealed enough variation among the genotypes and for the characters. Significant treatment and also interaction effect highlighted differential performance in two different water regimes. Osmotic stress experiments highlighted some genotypes as tolerant, some as susceptible and rest with average performance when compared against normal condition (Table 1). The genotype OEX 29 showed maximum percent increase for shoot length, root fresh weight, leaf fresh weight and minimum reduction shoot

Table 1. Best identified tolerant and susceptible genotypes on the basis of seedling characters in laboratory condition

S.No	Root length (cm)		Shoot length (cm)		Root fresh weight (g)		Root dry weight (g)		Shoot fresh weight (g)	
	G	% R	G	% R	G	% R	G	% R	G	% R
Five genotypes showing highest increment or minimum reduction										
1	OIN791	26.545	OEX29	13.748	OEX29	6.838	OIJ177	48.596	OEX 29	-1.679
2	OEX29	21.176	OIJ177	12.052	OIN791	6.753	OIN926	42.133	OIJ 177	-1.749
3	OIJ177	20.060	OIN791	11.986	OIJ177	4.260	OEX29	34.940	OIN 791	-2.106
4	OIJ218	19.229	OIJ266	9.828	KOM62	3.560	OIN791	34.424	OIN 976	-2.956
5	OIN937	17.684	OIN986	9.441	OIN378	2.521	OIN378	32.827	OIJ 266	-3.453
Five genotypes showing maximum reduction										
1	OIN 970	3.559	OIN 309	-44.127	JRO 8432	-58.128	OIJ 214	-79.151	OIJ 937	-61.426
2	JRO 524	2.548	CO 58	-35.522	OIJ 214	-58.000	JRO 524	-71.894	TJ 40	-51.037
3	OIJ 214	2.054	TJ 40	-34.998	JRO3690	-56.912	JRO 632	-71.200	B.RUPALI	-49.570
4	JRO 632	2.555	OIN 970	-34.029	OIN 970	-56.513	JRO3690	-70.597	S 19	-48.915
5	JRO3690	2.015	OEX 019	-31.596	JRO 524	-53.576	OIN 970	-70.039	CO 58	-48.485
CD@5%		**0.103**	**CD@5%**	**0.096**	**CD@5%**	**0.042**	**CD@5%**	**0.014**	**CD@5%**	**0.207**

Table 1. contd…

S.No	**Shoot dry weight (g)**		**Leaf fresh weight (g)**		**Leaf dry weight (g)**	
	G	**% R**	**G**	**% R**	**G**	**% R**
Five genotypes showing highest increment or minimum reduction						
1	OIN 926	-1.029	OEX 29	20.008	OIN378	38.514
2	OIN 791	-1.164	OIN581	15.735	OIN581	20.362
3	OEX 29	-1.575	OIN791	11.940	OIN791	17.805
4	JRO7835	-2.055	OIJ104	10.663	JRO878	14.815
5	OIJ 177	-2.104	OIJ177	10.553	OEX 29	13.380
Five genotypes showing maximum reduction						
1	OIJ 214	-59.077	OIJ 257	-49.279	OIJ 257	-55.882
2	OIN 714	-54.646	OIN 926	-49.219	JRO3690	-47.488
3	OIN 970	-53.928	JRO 524	-43.932	JRO 632	-46.577
4	OIJ 104	-53.659	OIJ 213	-43.774	JRO8432	-43.333
5	JRO 524	-53.542	OIN 427	-42.463	OIJ 214	-42.544
CD@5%		**0.017**	**CD@5%**	**0.055**	**CD@5%**	**0.009**

(G=Genotype, %R=Percentage of reduction)

fresh weight. The genotype OIN 791 showed minimum reduction for root length, OIJ 177 for root dry weight, tolerance index, OIN 926 for shoot dry weight, OIN 378 for leaf dry weight and these genotypes showed superior performance for all these character when subjected to moisture stress. Maximum reduction for root length was observed in OIN 970, shoot length in OIN 309, root fresh weight in JRO 8432, dry weight of root and shoot in OIJ 214, shoot fresh weight in OIJ 937 and dry and fresh weight of leaf in OIJ 257. In contrast to the present investigation, Ayodele and Fawusi (1990) reported significant reduction in root length due to moisture stress in all the genotypes. Garg and Singla (2004), Shiwachi *et al.* (2008) also reported reduction in total length of jute seedlings but the present investigation depicted increase of root length in eight genotypes due to their inherent capacity to tolerate stress. Under moisture stress environment, seven genotypes and nineteen genotypes showed significant increase in root fresh weight and dry weight, respectively. Maximum reduction in root fresh weight was noticed in JRO8432 and for root dry weight maximum reduction was noticed in OIJ 214 followed by

JRO 524 and JRO 632. None of the genotypes showed increase for fresh dry weight of shoot under stress. Maximum reduction of genotypes for shoot fresh weight were OIJ 937 followed by TJ 40 and shoot dry weight was OIJ 214 followed by OIN 714. Eight and thirteen genotypes showed increase in fresh and dry weight of leaf respectively. Maximum reduction of genotype for leaf fresh and dry weight was found to be OIJ 257. OIJ 177 showed highest tolerance index and OIN 378, OIN 791, OEX 29 had tolerance index very close to OIJ 177. Lowest tolerance index was evident OIN 970 followed by JRO 524. From the laboratory experiment, OEX 29, OIJ 177, OIN 791 could be considered as most tolerant genotypes at seedling stage (Table 4).

The seedling characters under field as well as pot also showed significant variations among genotypes in stress situation which provides enough scope for selection of moisture stress tolerance genotypes.

Field Experiment

Enhanced root length over control under moisture stress was evident in all the genotypes and maximum elongation was shown by OIJ 177, OIN 791, CO 58, Bidhan Rupali and OEX 29 and minimum elongation was shown by JRO 632, OIJ 214, OIN 970, JRO 3690 and JRO 524 (Table 2). Under stress, all genotypes had reduced root volume but least reduction was shown by four genotypes viz., OEX 29, OIJ 177, OIN 791 and S 19 which also showed maximum root elongation and while the genotypes which showed minimum root elongation had maximum reduction in root volume. Almost all genotypes except S 19, showed reduction in shoot length and minimum reduction was revealed by OIJ 177, OIN 791, OEX 019 while maximum reduction in JRO 3690, OIJ 214, JRO 632, JRO 8432. Maximum reduction for almost all seedlings characters like root dry weight, shoot fresh weight and dry weight, leaf fresh and dry weight was exhibited under water stress condition and three genotypes OEX 29, OIJ 177, OIN 791 showed minimum reduction for these characters. The genotypes JRO 632, JRO 8432 showed maximum reduction for all these characters and OIJ 214 for fresh and dry weight of root, shoot as well as leaf. Incremental effect on root fresh weight was noticed in ten genotypes, out of them three genotypes OEX 29, OIN 791 and OIJ 177 showed maximum enhanced effect for the same. On the other hand, genotypes OIJ 214, JRO 524, JRO 3690, JRO 8432, JRO 632 also showed maximum reduction in root fresh weight.

Pot experiment

Enhanced effect as a result of exposure to moisture stress was evident in pot culture for root length, root volume, root fresh weight, root dry weight and shoot length in seven, eight, eight, four and two genotypes respectively (Table 3). All the genotypes showed maximum reduction for shoot fresh and dry weight of shoot and leaf. The genotypes, OIN 791, OIJ 177 and OEX 29 consistently showed maximum enhancement effect or minimum reduction effect due to moisture stress. Genotypes JRO 3690, JRO 632 and OIN 970, JRO 8432 and JRO 524 were badly suffered genotypes under moisture stress and were also found as most affected genotypes for many of the seedlings characters. Tolerance index of OIJ 177, OIN 791, and OEX 29 was found to be consistently superior in field and pot experiments too and JRO 8432, JRO 3690, JRO 524 showed least tolerance index (Table 4).

Tolerance index from all these set of experiments showed significant correlations with all other seedling characters except for shoot length, root fresh weight, shoot fresh weight and the characters also showed positive significant inter-se relationship. Selection at seedling stage on the basis of these characters will help to identify high fibre yielding genotypes at maturity. Under field condition, tolerance index proved as an important selection criteria as it showed significant associations with all the seedling characters. Root volume, root fresh weight, fresh and dry weight of shoot and leaf showed significant inter-se relations and could be favourably considered for selection of moisture stress tolerant genotypes. Tolerance index along with a few other seedling characters could be proved useful for isolation of moisture stress tolerant genotypes.

On the basis of tolerance index and other seedling characters genotypes OIJ 177, OIN 791 and OEX 29 could be considered as the most tolerant and JRO 8432, JRO 3690 and JRO 524 as the most susceptible under moisture stress environment. These tolerant genotypes could be recommended as varieties to be grown under moisture stress environment as they will maintain their growth satisfactory under such environment. Further these genotypes can be utilized for improvement of other high yielding genotypes.

Table 2. Best identified tolerant and susceptible genotypes on the basis of seedling characters in field condition

S.No	Root length (cm)		Root volume (cc)c		Root fresh weight (g)		Root dry weight (g)		Shoot length (cm)	
	G	% R	G	% R	G	% R	G	% R	G	% R
Five genotypes showing highest increment or minimum reduction										
1	OIJ177	31.910	OEX29	-5.333	OEX29	13.424	OEX 29	-12.967	S 19	14.978
2	OIN791	31.389	OIJ177	-5.684	OIN791	12.422	OIN 791	-13.989	OIJ 177	-5.809
3	CO 58	30.788	OIN791	-8.759	OIJ177	9.715	OIJ 177	-15.821	OIN 791	-7.486
4	B.RUPALI	30.615	S19	-10.204	OIN 990	7.061	OIN 990	-19.561	OEX019	-8.792
5	OEX 29	30.142	JR0128	-10.435	OEX014	5.958	OEX014	-20.210	OIJ 263	-8.796
Five genotypes showing maximum reduction										
1	JRO 632	4.329	OIN970	-33.784	OIJ 214	-37.891	OIJ 214	-51.853	JRO3690	-46.653
2	OIJ 214	5.367	JRO632	-28.816	JRO 524	-35.414	JRO3690	-51.130	OIJ 214	-45.895
3	OIN 970	5.985	OIJ 214	-28.369	JRO 3690	-35.269	JRO 524	-50.650	JRO 632	-42.740
4	JRO 3690	6.156	JRO524	-28.169	JRO 8432	-32.968	JRO8432	-48.308	JRO 524	-42.688
5	JRO 524	6.453	JRO8432	-23.188	JRO 632	-31.617	JRO 632	-48.050	JRO8432	-40.673

Table 2. Cond. .....

S.No	Shoot fresh weight (g)		Shoot dry weight (g)		Leaf fresh weight (g)		Leaf dry weight (g)	
	G	% R	G	% R	G	% R	G	% R
Five genotypes showing highest increment or minimum reduction								
1	OEX29	-2.689	OEX 29	-4.456	OIN791	-10.608	OIN791	-9.687
2	OIJ177	-3.037	OIN581	-5.880	OIJ177	-11.423	OIN976	-10.164
3	OIN791	-3.442	OIN791	-6.746	OEX29	-12.715	OIN409	-10.277
4	OIN082	-10.070	OIJ104	-12.918	OIN975	-15.517	CO 58	-10.300
5	JRO878	-10.499	OIJ177	-13.906	OIN976	-15.721	OIJ213	-11.079
Five genotypes showing maximum reduction								
1	JRO 632	-46.769	OIJ 257	-69.611	OIN 970	-52.213	JRO 524	-55.643
2	JRO8432	-45.085	OIN 926	-57.197	JRO 524	-44.227	JRO 632	-53.807
3	JRO3690	-36.373	JRO 524	-55.017	OIJ 214	-43.209	OIJ 214	-52.316
4	OIN 970	-35.212	OIJ 213	-52.131	JRO8432	-41.782	JRO 8432	-50.784
5	OIJ 214	-34.375	OIN 427	-51.762	JRO 632	-40.984	JRO 66	-48.187

(G=Genotype, %R=Percentage of reduction)

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Table 3. Best identified tolerant and susceptible genotypes on the basis of seedling characters in pot condition

S.No	Root length (cm)		Root volume (cc)		Root fresh weight (g)		Root dry weight (g)		Shoot length (cm)	
	G	% R	G	% R	G	% R	G	% R	G	% R
Five genotypes showing highest increment or minimum reduction										
1	OIN 791	16.556	OIN 791	44.275	OIJ 177	13.636	OIN791	6.311	OIJ 177	3.779
2	OEX 29	13.180	OIJ 177	37.209	OEX 29	10.145	OEX29	6.207	OEX 29	0.567
3	OIJ 177	9.781	OEX 29	31.163	OIN 791	8.955	OIJ177	3.280	OIN 791	-2.154
4	OIJ 263	7.729	OIJ 263	18.532	OIJ 263	7.759	OIJ218	1.850	JRO 878	-8.168
5	OIJ 218	1.855	OIJ 218	16.216	OIN 259	4.669	OIJ263	-3.073	OIJ 264	-9.125
Five genotypes showing maximum reduction										
1	JRO 632	-51.029	OIN 981	-52.038	JRO3690	-32.362	JRO3690	-34.850	OIN 970	-58.588
2	OIN 970	-44.694	JRO 632	-43.243	JRO8432	-27.351	OIN 970	-33.600	JRO3690	-43.612
3	JRO3690	-42.547	OIN 970	-42.581	JRO 524	-27.333	JRO8432	-30.526	OIJ 214	-42.206
4	OIN926	-42.221	JRO 524	-40.268	OIJ 214	-27.167	JRO 632	-30.444	OEX 019	-39.532
5	JRO524	-41.188	JRO3690	-40.268	JRO 632	-26.936	OIJ 214	-30.432	JRO 632	-39.084

Table 3.

S.No	Shoot fresh weight (g)		Shoot dry weight (g)		Leaf fresh weight (g)		Leaf dry weight (g)	
	G	% R	G	% R	G	% R	G	% R
Five genotypes showing highest increment or minimum reduction								
1	OIJ 177	-1.127	OIJ177	-6.734	OEX 29	-1.146	OEX 29	-7.945
2	OEX 29	-2.849	OEX29	-8.279	OIJ 177	-1.780	OIJ 177	-8.483
3	OIN 791	-5.042	OIN791	-10.238	OIN 791	-3.794	OIN 791	-10.280
4	OIN 515	-8.465	OIN515	-13.223	OIN 533	-5.322	OIJ 213	-11.664
5	OIN 976	-9.325	OIN976	-14.083	OIJ 213	-5.431	OIN 533	-11.681
Five genotypes showing maximum reduction								
1	JRO3690	-50.886	JRO3690	-51.110	JRO 632	-61.164	JRO 632	-60.684
2	JRO 632	-43.819	JRO 632	-44.871	JRO3690	-60.177	JRO 3690	-59.465
3	JRO 524	-43.490	JRO 524	-44.340	JRO8432	-59.396	JRO 8432	-59.112
4	JRO8432	-42.925	JRO8432	-44.318	JRO 524	-58.926	JRO 524	-58.498
5	OIN 970	-42.619	OIN 970	-44.293	OIN 970	-57.697	OIN 970	-57.865

(G=Genotype, %R=Percentage of reduction)

Table 4. Tolerance index in laboratory, field and pot condition

Laboratory condition TI= TDWT/ TDWC*100		Field condition TI= TDWR/ TDWI*100		Pot condition TI= TDWR/ TDWI*100		Laboratory condition TI= TDWT/ TDWC*100		Field condition TI= TDWR/ TDWI*100		Pot condition TI= TDWR/ TDWI*100	
Five genotypes showing highest TI						Five genotypes showing lowest TI					
G	TI	G	TI	G	TI	G	TI	G	TI	G	TI
OIJ 177	117.804	OIN 791	93.604	OIJ177	94.152	OIJ 214	35.456	JRO 8432	36.228	JRO 3690	48.441
OIN 378	114.268	OIJ 177	91.833	OEX29	94.139	JRO 8432	42.551	JRO 524	44.067	JRO 632	51.037
OIN 791	113.258	OEX 29	91.585	OIN791	92.189	JRO 3690	42.563	OIJ 214	46.104	JRO 8432	51.892
OEX 29	112.969	OIN 976	86.561	JRO878	83.697	JRO 524	42.753	JRO 632	48.032	OIN 970	51.966
OIN 581	106.926	OIN 986	83.780	OIN915	82.813	OIJ 257	43.192	JRO 3690	50.917	JRO 524	52.374
Mean	80.536		68.862		71.669		80.536		68.862		71.669
CD@5%	3.430		2.653		2.702		3.430		2.653		2.702

(G=Genotype, TI= Tolerance Index, TDWT= Total Dry Weight of Treatment, Total Dry Weight of Control, TDWR= Total Dry Weight of Rainfed condition , TDWI= Total Dry Weight of Irrigated condition)

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Genetic Analysis of Staygreen Trait and its Association with Morpho-physiological Traits under Water Deficit Stress in Wheat

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The present study was conducted to assess the relation of staygreen trait with yield attributes and physiological traits under water deficit condition in wheat and to identify genetically diverse and agronomically desirable functional staygreen parents for incorporation in the breeding programmes for the development of drought tolerant genotypes. Heritability estimates along with genetic advance are high for leaf senescence rate (LSR). Present study also demonstrated significant positive association of staygreen trait LSR with RWC, photosynthetic rate, ear weight, thousand kernel weight and grain yield. Grouping genotypes for drought tolerance and staygreen trait suggested, CHIRYA7 as the most drought tolerant (DSI = 0.58) staygreen genotype (LSR=0.13) and CBW38 as drought susceptible (DSI = 1.12) fast senescing (0.48) genotype. D^2-clustering grouped the genotypes into six clusters on the basis of similarity in the traits studied. Cluster III genotypes display staygreen character because it exhibited maximum cluster mean for photosynthetic rate, grain yield, test weight, SCMR, ear weight, biological yield, RWC and minimum cluster mean for DSI and LSR. There is a maximum intercluster distance between Cluster III and cluster VI hence staygreen genotypes from cluster III viz. CHIRYA7, HW2041 can be selected and crossed with cluster VI genotypes viz. HW2063 for incorporation of functional staygreen trait for development of drought tolerant genotypes. The clustering of genotypes will help to identify divergent genetic material for obtaining highly heterotic functional staygreen wheat hybrid for drought condition.

Key Words: D^2 clustering, Leaf senescence, Staygreen trait, DSI, Wheat, Drought

Introduction

Wheat *(T. aestivum L. em Thell.)* is a staple food for more than 35% of the world population and covers 30% of the world's cereal producing area. Abiotic stress affects 220 million hectare of wheat producing area worldwide which consequently lowers its yield (Cossani *et al.*, 2012). Wheat yields have been reported to reduce by 50-90% of their irrigated potential by drought (Reynolds *et al.*, 2005) Thus, breeding for drought tolerance is a major objective in crop improvement programme. Traits associated with post flowering drought tolerance include improved rooting depth (Sharp *et al.*, 2004), stay-green (Rajcan and Tollenaar, 1999; Borrell *et al.*, 2000), longer grain filling duration, increased grain filling rate and increased individual grain weight (Harris *et al.*, 2007). Drought leads to premature leaf senescence which decreases leaf area duration consequently resulting in yield reduction. Senescence rate in wheat is particularly sensitive to water and heat stress and like many other traits, genetic variation for this trait has been reported (Falqueto *et al.*, 2009; Srivalli and Chopra, 2009). Genotypes possessing the ability to maintain green leaf area duration throughout grain filling are termed as "staygreen"(SG) and are potential candidates to assure yield in semi-arid regions. SG genotypes have been used successfully in sorghum for yield stability and promises as a selection tool in wheat (Christopher *et al.*, 2008). Therefore, selection of slow senescing genotypes with high yield stability under drought condition should be the selection criterion for functional staygreen genotypes. Modern cultivars in wheat and other crops are often genetically similar, with a rather narrow genetic base. The presence of genetic diversity is important for successful wheat breeding because artificial crossing among dissimilar parents allows segregation and recombination of different favourable alleles (Bered *et al.*, 2002). A wide array of material including wheat land races, local varieties, advanced lines, and crosses with ancestral species are being used by different wheat breeding programmes in the country to generate the necessary genetic diversity. Better understanding of the genetic basis of this variability will improve the efficiency of

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wheat improvement for drought tolerance. The present study was undertaken to identify genetically diverse and agronomically desirable functional staygreen parents for developing high yielding functional staygreen wheat genotypes under drought condition.

Materials and Methods

During *Rabi* (2012-2013) a pot culture experiment was conducted on thirty five core elite wheat germplasm from India and CIMMYT, Mexico. Sowing was done in earthen pots (about 30 cm in diameter and 30 cm in depth) with four plants per pot filled with clay loam soil and farmyard manure (FYM) in 3:1 ratio during *rabi* (winter) season. Nitrogen, phosphorus and potash fertilizers were applied at the rate of 60: 60: 60 kg per hectare, respectively in the form of urea, single super phosphate and muriate of potash at the time of sowing. Remaining 60 kg N ha^{-1} was given after 25 days of sowing. Plants were subjected to water deficit stress for eight days after anthesis (DAA) by withholding irrigation (RWC 65-70%), while in irrigated plants RWC ranged from 80-85%. The response of plants in terms of growth and physiological traits were studied in upper most fully expanded flag leaf.

Photosynthetic Rate

Leaves were categorized into green and yellow/dead, and the rate of photosynthesis was measured using portable Infrared Gas Analyser (IRGA), Model LI-6400XT (Li-COR Ltd., Lincoln, Nebraska, USA) by operating it in the closed mode between 10.00-11.00 a.m. when relative humidity, temperature, photosynthetic photon flux density and CO_2 concentration ranged from 50-60%, 30 to 35^oC, 1200 µmol $m^{-2}s^{-1}$ and 350 to 360 µmol mol^{-1}, respectively. Fifteen flag leaves per treatment were selected at random for photosynthetic rate measurement and expressed in µmol CO_2 m^{-2} s^1.

SPAD Chlorophyll Meter Reading (SCMR)

Soil and plant analyser development (SPAD) values were measured in the middle part of flag leaves using portable Minolta SPAD-502 chlorophyll meter (Minolta camera Co. Ltd., Osaka, Japan) after flowering at the end of stress period for eight days. The average reading of 10 leaves per pot was recorded and used in the analysis. Measurements were carried out twelve times between flowering and the end of senescence on three flag leaves for each genotype.

Leaf Senescence Rate

Phenotyping for LSR was done visually and senescence score was estimated, dividing the percentage of estimate area that is dead by time duration in days (Lu *et al.*, 2011). The twelve dates of assessments were expressed as ($\Sigma t_1 - \Sigma t_{12}$) and the corresponding senescence score ($S_1 - S_{12}$). The average senescence (S_a) was calculated after 20 days from 50% anthesis as ($S_i+_{20} - S_i$) / ($\Sigma t_i+_{20} - \Sigma t_i$) as given by (Guendouz et *al.*, 2012).

Yield and Yield Components

The plants were harvested separately from control and water stressed pots. Measurements on grain yield per plant were recorded as economic yield. The whole plant dry weight was measured as biological yield. Three replications were taken for each parameter.

Drought Susceptibility Index (DSI)

The drought susceptibility index was calculated by using the formulae of Fisher and Maurer, 1978.

$$S = (1-Y/Yp)/D$$

Where S is drought susceptibility index, D is stress intensity, Y is yield under stress, Yp is yield without stress and

$$D = \text{Stress intensity} = 1-X/Xp$$

Where X and Xp represent average varieties yield under stress and non-stress conditions respectively. The S was used to characterize the relative drought stress tolerance of the various species ($S \leq 0.50$ high drought tolerant, $S \geq 0.50 \leq 1.00$ moderately stress tolerant and $S > 1.00$ Susceptible).

Statistics

The data obtained from the experiments was subjected to analysis of variance (ANOVA) by complete randomized design and F-test was carried out to test the significance of the treatment differences. The correlation was done using MS Office Excel. The genetic divergence was estimated by using D^2 statistics of Mahalanobis, 1936, and the grouping of the genotypes into clusters was done using Euclidean method of clustering. The INDOSTAT software version 9.2 was utilized for statistical analysis.

Result and Discussion

The analysis of variance was highly significant among the divergent genotypes for all the morpho-physiological

traits under study, which revealed the presence of considerable variability among the studied genotypes.

Genetic Analysis

Genotypic coefficient of variation, phenotypic coefficient of variation, heritability (broad sense), genetic advance and genetic advance expressed as percent of mean for 12 characters are shown in Table 1. The PCV values were higher than GCV values for all the traits that reflects the influence of environment on traits. The estimates of GCV and PCV were high for all the characters studied except SCMR value, relative water content and plant height which showed the moderate GCV and PCV (Table 1). High heritability coupled with high genetic gain for LSR indicates that it exhibits additive gene effect and is not influenced by environmental effects which is in agreement with Chen *et al.,* (2013) who showed that the broad-sense heritabilities of staygreen trait such as ratio of visible green leaves at physiological maturity in maize were relatively high. It indicates that selection may be effective in early segregating generation for these traits under water deficit. Therefore, there seems a scope for improvement in these traits or selection would be effective in water deficit condition.

Genetic Correlation Studies

LSR shows significant negative correlation with SCMR (-0.59) indicating that drought induced leaf senescence leads to reduction in chlorophyll (Table 2). Likewise, Guendouz *et al.,* (2012) also reported significant negative correlation between chlorophyll content and average senescence (r = -0.68). Chlorophyll is the major photosynthetic pigment in plants which functions to capture and transfer light energy. A functional staygreen is defined as retaining both leaf greenness and photosynthetic competence much longer during leaf senescence, while non-functional staygreen is defined as maintaining only leaf greenness (Thomas and Smart, 1993; Thomas and Howarth, 2000). LSR shows positive association with the photosynthetic rate established by the fact that it shows significant negative correlation with the photosynthetic rate which is in agreement with Patro *et al.*, (2014) who showed that during dehydration induced leaf senescence in *Arabidopsis thaliana* leads to reduction in photosynthesic rate due to decrease in primary photochemical reaction of thylakoids. SG trait also show significant correlation and positive association with yield determining components such as ear weight per plant, 1000 kernel weight, biological yield and harvest index under drought (Table 2). This positive association of SG trait with other yield attributes consequently lead to its significant correlation (LSR= -0.49) with grain yield under drought condition. These positive association of staygreen trait with ear weight and grain yield is positive which is in agreement with Distelfeld *et al.,* (2014) who illustrated that whole-plant senescence overlaps with grain filling, and the synchronization of these two processes is highly important in determining yield, particularly through the grain weight component. It is evident from this positive association of staygreen trait with test weight which was supported by the observations of Spano *et al.*, (2003) that 'staygreen' mutants of durum wheat maintained photosynthetic competence for longer time than the parental line, attained a higher grain weight. LSR show significant significant negative correlation with drought susceptibility index (DSI) which is in agreement with Jordan *et al.,* (2012) who showed that staygreen trait is positively correlated with sorghum grain yield in field conditions under terminal drought.

Table 1. Estimates of genetic parameters for 12 quantitative characters of 35 wheat genotypes.

Characters	GCV	PCV	h^2 (bs)	GA	GG
LSR	33.020	34.320	93.069	0.205	65.624
SCMR	6.321	12.150	27.036	0.300	6.764
Pn rate	60.264	62.061	94.261	8.596	90.514
RWC	7.660	9.771	61.469	6.611	12.377
PHT (cm)	10.481	14.361	53.302	12.664	15.763
TPP	32.400	34.680	87.277	5.209	62.359
EWP (g)	47.021	49.371	90.672	7.544	92.223
TKW (g)	33.472	36.040	86.269	19.223	64.047
BYP (g)	32.871	36.541	80.957	11.561	60.933
HI (%)	33.961	36.320	87.456	16.157	65.431
GYP (g)	50.291	51.923	93.840	4.746	100.361
DSI	20.262	20.371	98.943	0.417	41.508

GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, h^2 = heritability, GA= Genetic Advance & GG= Genetic gain

Classification of genotypes on the basis of average leaf senescence rate and drought susceptibility index

Genotypes for staygreen trait were selected on the basis of LSR and (DSI). The mean value of LSR and DSI. The mean value of DSI and LSR was 0.31 and 1.00 respectively. There are ten genotypes with less than average LSR and DSI and these were identified as functional staygreen genotypes (Fig. 1), 13 genotypes with more than average LSR and DSI were identified

Table 2. Correlation of staygreen trait with other drought tolerance and yield attributes in wheat under drought condition

Character	LSR	SCMR	Pn rate	RWC	PHt	TPP	EWP	TKW	BY	HI	DSI
LSR	1.0										
SCMR	-0.59***	1.0									
PR	-0.33*	0.87****	1.00								
RWC	-0.97****	0.69****	0.48***	1.0							
PH	0.18*	0.13^{ns}	0.07^{ns}	-0.09^{ns}	1.00						
TPP	0.06^{ns}	0.26^{ns}	0.24*	0.04^{ns}	0.33	1.00					
EWP	-0.61****	0.73****	0.40***	0.80****	0.05^{ns}	0.39*	1.00				
TW	-0.52***	0.59***	0.61****	0.65*****	-0.27*	-0.10^{ns}	0.45**	1.00			
BY	-0.35*	0.73****	0.43***	0.42***	0.32*	0.66****	0.71****	0.13^{ns}	1.00		
HI	-0.34*	0.15^{ns}	0.01^{ns}	0.37**	0.17*	-0.28^{ns}	0.25^{ns}	0.38*	0.02^{ns}	1.00	
DSI	0.54***	-0.61****	-0.21^{ns}	-0.66****	-0.37**	-0.14^{ns}	-0.67****	-0.45**	-0.53***	-0.63****	1.00
GY	-0.50**	0.66****	0.37*	0.64****	0.31**	0.16ns	0.67****	0.51***	0.55***	0.65****	-0.85*****

**** means significance at 0.01% level of significance, *** means significance at 0.1 % level of significance, ** means significant at 1% level of significance, * means significant at 5% level of significance, ns means non significant.
LSR= Leaf senescence rate, GI=Grenness index, Pn rate=Photosynthetic rate, RWC=Relative Water content, PHt=Plant height, TPP= tillers per plant, EWP=Ear weight per plant, TKW=1000 kernel weight, BYP=Biological yield per plant, HI=Harvest index, GYP= grain yield per plant, DSI= Drought susceptibility index.

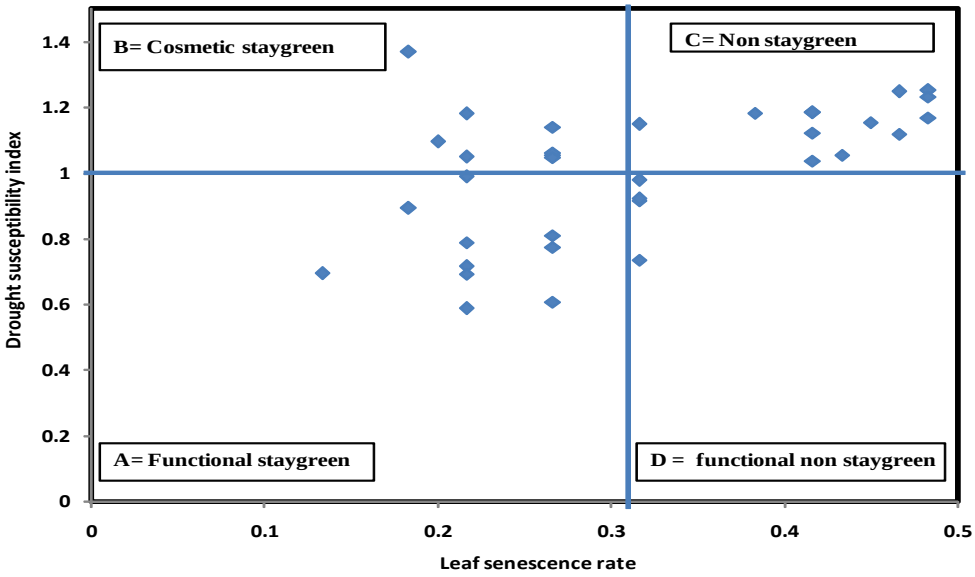

Fig. 1. Genotypes Classified on the basis of average LRS watered and DSI under water deficit condition. (A) identifies functional staygreen genotypes exhibiting low LSR and low DSI. (B) identifies non-functional staygreen genotypes (Cosmetic staygreen genotypes) exhibiting low LSR and high DSI. (C) identifies non-staygreen genotypes exhibiting high LSR and high DSI. (D) identifies genotypes exhibiting High LSR and Low DSI (functional non-staygreen).

as non-staygreen genotypes, eight genotypes with low LSR and high DSI were identified as cosmetic staygreen genotypes, and four genotypes with high LSR and low DSI were identified. Out of ten functional SG genotypes, CHIRYA7 and HW2041 have been selected as agronomically desirable functional staygreen parents with maximum SCMR, photosynthetic rate, grain yield and grain weight. This is in agreement with (Christopher *et al.*, 2008; Gous *et al.*, 2013) who shows that staygreen mutants in various wheat cultivars have increased grain weight and yield as a result of delayed senescence and also perform better under water deficit stress.

Genetic Diversity Analysis

On the basis of Mahalanobis D^2 following Euclidean method for clustering, thirty five genotypes were grouped

Table 3. Distributing pattern of 35 genotypes of wheat into six clusters based on Euclidean analysis

Cluster group	No. of genotypes	Name of genotypes
Cluster I	6	PBW373, HW2060, HW2055, HW2008, HW 2085, HD2894
Cluster II	4	HW2061, HW2027, HW4010, HW2042
Cluster III	4	CHIRYA7, HW2041, HW4024, PBW502
Cluster IV	9	PBW555, CHIRYA3, CHIRYA1, LOK-64, HW2020, HW4050, GW-322, HW4030, HW4009
Cluster V	11	CBW-38, HW2033, UP-2696, HW4060,WL711, HW2080, HW2051,HW4007,Shanghai1, HW4203, HD2789
Cluster VI	1	HW 2063

Table 4. Average intra and inter cluster D^2 values for 35 genotypes of wheat

	1 Cluster	2 Cluster	3 Cluster	4 Cluster	5 Cluster	6 Cluster
Cluster I	118.918	283.136	306.899	385.231	601.232	1133.336
Cluster II		167.568	344.647	280.79	318.263	708.719
Cluster III			150.869	731.943	886.635	1382.667
Cluster IV				96.694	157.141	373.980
Cluster V					93.086	280.959
Cluster VI						0.000

into six clusters with cluster-wise variable number of genotypes (Table 4 Fig. 2) developed by various centres and located at different geographical locations. A distribution pattern of all the genotypes into various clusters (Fig. 2) showed the presence of considerable genetic divergence among the genotypes for most of the studied traits. Amongst six clusters, cluster VI was mono-genotype cluster, whereas, cluster V was the largest having 11 genotypes involving varieties/ strains from various centres. Similarly, cluster II and III each consisted of four genotypes and cluster I and IV consisted of nine genotypes. Amasiddha *et al*., (2013) also investigated genetic diversity for moisture deficit stress adaptive traits in bread wheat and classified the 33 genotypes of into six clusters. It was observed that intra cluster distance was maximum for cluster II (167.56) followed by cluster III (150.86) and cluster I (118.91), while cluster IV (96.69) and cluster V (93.08) had slightly lower values of intra-cluster distance. Thus, genotypes included within the cluster I, II and III revealed maximum diversity. The highest inter-cluster distance was noted between cluster III and cluster VI which is followed by cluster I and VI (1133.33), III and V (886.63) and cluster III and IV (731.94). Maximum cluster mean for the character grain yield per plant was observed for the cluster III (8.677). Cluster III, was selected for higher value of SCMR, photosynthetic rate, RWC, ear weight per plant, test weight, harvest index and grain yield. Cluster V has lowest RWC, Ear weight and test weight and low biological yield, grain yield, harvest index and categorized as fast senescing and drought susceptible due to maximum LSR (0.421) and DSI (1.183). Hence, cluster III genotypes can be categorized as functional staygreen exhibiting minimum cluster mean. for LSR and DSI. All the 10 genotypes out of 11 present in cluster V overlapped with the list of non-staygreen genotypes classified (Fig. 1). Hence, cluster V can be categorized as non-staygreen cluster exhibiting maximum cluster mean for LSR and DSI. Intra and inter-cluster distances (table 4) were used to identify genetically diverse functional SG parents that could be recommended for incorporation in the breeding programmes for the development of SG genotypes. Inter cluster distance between Cluster III and cluster VI is maximum, hence for the development of functional SG varieties in wheat under water deficit condition, the genotypes from cluster III viz., CHIRYA7, HW2041 can be selected and crossed with cluster VI genotype viz. HW2063.

Conclusions

Staygreen trait i.e., leaf senescence rate shows positive association with relative water content, photosynthetic rate, ear weight, thousand kernel weight and grain yield. Grouping genotype for drought tolerance and staygreen trait suggested, CHIRYA7 as the most drought tolerant staygreen genotype and CBW38 as drought susceptible fast senescing genotype. D^2-clustering grouped the genotypes into six clusters and cluster III genotype displayed minimum cluster mean for DSI and LSR.

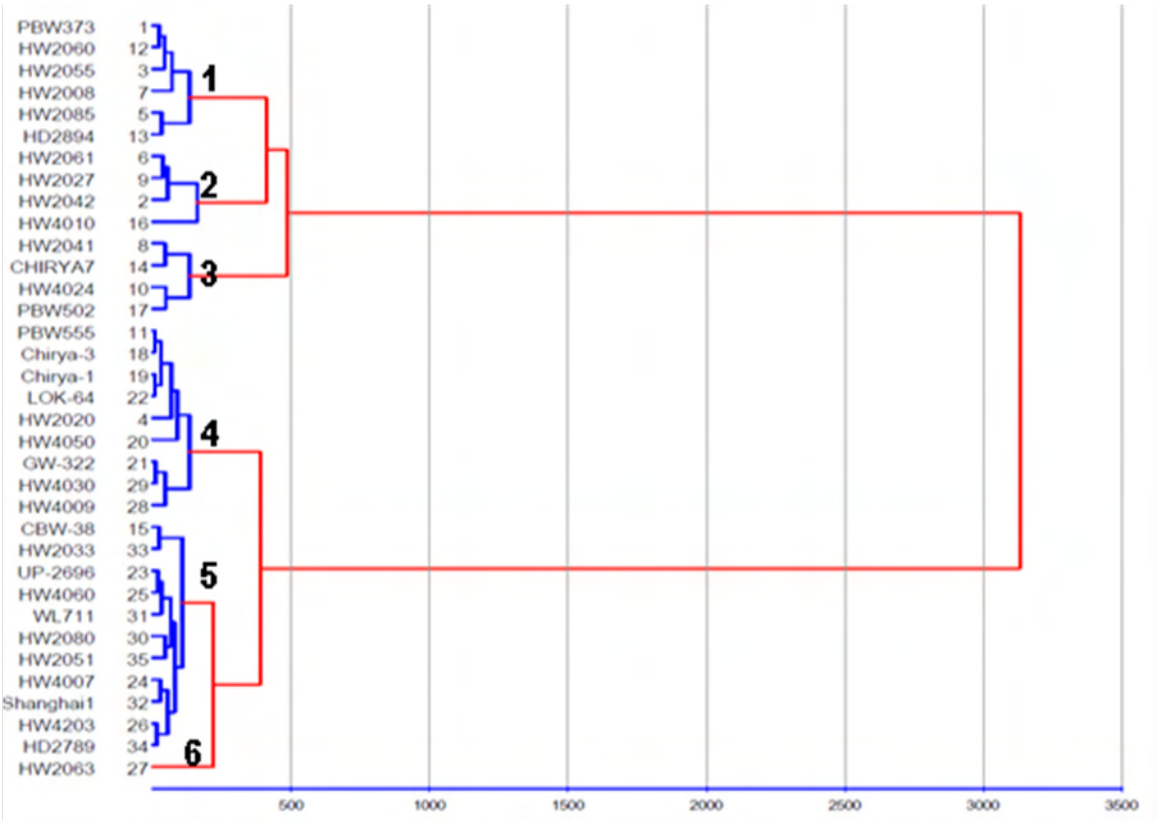

Fig. 2. K Wards minimum variance dendrogram which shows the distribution of the 35 wheat genotypes under drought condition

Maximum inter-cluster distance was between Cluster III and cluster VI hence staygreen genotypes from cluster III viz. CHIRYA7 and HW2041 can be selected and crossed with cluster VI genotype i.e., HW2063 for incorporation of functional staygreen trait for development of drought tolerant genotypes. The genotypes identified will be used as donors in conventional/molecular breeding programme for the development of drought tolerant wheat genotypes.

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The Report of *Curcuma kshonapatra* (Zingiberaceae) from Karnataka, India

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(Received: 07 October 2014; Revised: 04 December 2015; Accepted: 29 December 2015)

Attempt to explore and collect resources of germplasm of wild relatives of turmeric in India resulted in amassing of a total of 123 samples belonging to 11 accepted species and few unidentified entities of the section *Nontuberosa* of the genus *Curcuma*. One entity from Udupi, Karnataka happened to be distinct from its close ally *C. albiflora* Thwaites in having tall thin plant type, purple tint on leaf blade, dark purple spikes, highly exerted and showy flowers, is being reported therefore reported as a new species with some notes here.

Key Words: *Curcuma kshonapatra,* New species, *Nontuberosa*, Section, Taxonomy

Introduction

A total of 123 germplasm collections of the sect. *Nontuberosa* (Velayudhan *et al*. 1996) of the genus *Curcuma* L. were made–by NBPGR (ICAR) under the mission mode project on National Agricultural Technology Project (Plant Biodiversity) from various parts of India during 1999-2005. Collections from South-western coastal and hilly region of India included 11 presently accepted species (WCSP, 2014) and some unidentified entities. One of the entities was very distinct and was for years under studied *in situ* as well as *ex situ*. It can be grouped along with the white staminode bearing species of the genus such as *C. albiflora* Thwaites, *C. oligantha* Trimen, *C. karnatakensis* Amalraj, Velay. & Mural. and *C. mutabilis* Skornick. M. Sabu & Prasanthk; which were reported from Western Ghats. It is found to be associated with a large population of a dominant species which is suspected as *C. albiflora* Thwaites (Plate 1). It is being described here as a new species due to some characteristic features such as erect and thin tall plant type with few suckers that bear elliptic/ lanceolate leaf lamina without wavy margin and slender purplish petioles as against more robust and profuse suckering companion species. Leaf lamina has purple midrib and closely arranged prominent veins which radiates dark purple wash or purple violet tint below the juvenile leaves, which fade at maturity. The key character of the species i.e. purple midrib, is perhaps first of its kind in species (sect. *Nontuberosa*) having no sessile fingers from India. Usually, this character purple midrib is associated with the presence of sessile finger on the root-stock in the genus and hence it appears to have an evolutionary significance and is associated with a group of sessile tuber bearing species under the genus *Curcuma* such as *C. zedoaria* (Christ.) Roscoe, *C. zanthorrhiza* Roxb; *C. ferruginea* Roxb; *C. comosa* Roxb; *C. latifolia* Roscoe; *C. amarissima* Roscoe, etc., falling under the sect. *Tuberosa* Velay., Amalraj & Mural. (Velayudhan et al. 1996). As the present species obviously falls under the sect. *Nontuberosa.* The purple colouration in the midrib region of the leaf and the purple tint of the tender leaves and petiole, purplish flower bracts, highly exerted and beaked flower buds are taken as the key character to delimit the present species from all other closely related species with white staminodes to propose a specific epithet '*kshonapatra*' which in Sanskrit means reddish purple tint on leaf similar to that of 'kshona' meaning blood.

C. albiflorae Thwaites, *C. oliganthae* Trimen, C. *karnatakensis* Amalraj, Velay. & Mural. *C. mutabilis* Skornick. M. Sabu & Prasanthk. *C. inodora* Blatt., characteribus vegetativorum aeriorum; absentia dactylorum sessilium; floribus maxime exertis et staminodiis albis arte affinis, sed costa folii purpurea; basi folii juvenis purpureo- tincta; spicis magnis, compactis, globosis ad ovalibus clare differt.

Root–stock conical to cylindrical, 7.2 x 1.3 cm branched with one or two secondary (lateral) mother rhizomes bearing suckers which resemble mother rhizome in shape but smaller, rhizome cortex flesh whitish cream, core pale yellow, flesh odourless, taste slightly astringent, less aromatic in smell; roots terminating into stipitate tubers, 10-15 in number, fusiform, rarely beaded, 3.0-3.5 cm x 2.0-2.2 cm. in size, cortex dirty or transparent

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a) *Curcuma kshonapatra* (Lateral spike)

b) *Curcuma kshonapatra* (Lateral spike with early leaves)

c) Associated plant type in the site

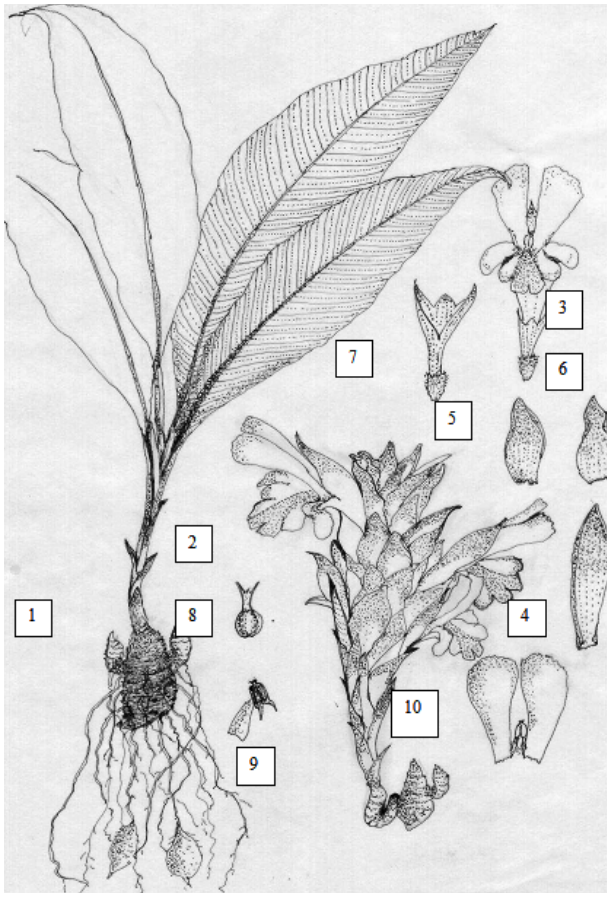
d) *C. shonapatra* lateral spike with beaked flower buds

e) Mature plant of *C. kshonapatra*

f) Root stock of *C. kshonapatra*

Plate 1 *C. kshonapatra* showing early plant type, lateral spikes and root stock along with the associated species

white, core white, without any smell and taste; plants erect, reaching up to 70 cm tall, bearing 2-4 suckers; petiole erect to semi-erect, 25-55 cm, sheath 15-30 cm long, margin purple extending to the leaf base, glabrous, ligule purple, pseudo-stem with 5-6 scale leaves at the base, 2.0 to 15.0 cm long; leaves 5-6, semi-erect to prostrate and drooping above middle at maturity, lamina 25-30 x 9-11 cm, elliptic – lanceolate, margin less wavy in tender ones and highly wavy in mature ones, veins arise from the midrib at 45^{o} running closely parallel up to the margin, highly pronounced, young petiole with reddish tint, midrib is purple throughout along the channel spreading towards the leaf visible on the dorsal side towards the base especially in younger ones, base gradually tapering or acute, rarely oblique, acute-acuminate at the tip, obtuse or rounded at the base, green with reddish purple tint above middle, glabrous. Spike either lateral or central, the former arising from the side buds developing from the main root-stock or branches towards the top, each bud bifurcating into two, one being the inner one to form a spike and the other outer one which is usually in bud stage at the time of blooming to form a leafy shoot during the main season. Those associated with the shoot form scale leaves at the base and the latter borne central spike arising from the tuft of leaves during the main growing season. Spike 9.0-12.5 cm long without coma, oblong and compact with greenish purple or purple flower bracts bearing highly exerted and showy flowers with white staminodes. It is showy, oval to oblong, varies in size with age of the plant; peduncle 8-12.0 cm long, covered by whitish scaly bracts that are oblong to ovate towards the tip, sterile bracts vary in length (2.5 cm in the case of lower ones and 6 cm in the upper ones and width from 1.5 to 1.6 cm), whitish at the base tinged with red, purple or pink towards the top, glabrous, no coma bracts seen (usually flower buds arise from axils of all bracts)-, flower bracts 4-5 in number, shape vary from ovate to slightly oblong, narrow linear towards top with acute tip, purple violet towards tip, bracts hairy on both sides, length varying 2.2 to 6.5 and width from 1.5- 1.6 cm, flowers 2 in a bract, highly exerted up to 7 cm long, buds whitish with light rose tinge and prominently beaked, calyx up to 2 cm long, hairy, three toothed, one side deeply cleft, middle tooth ovate and acute and shorter than the other lobes which are longer and acuminate, with purple tint, corolla tube up to 3.5 cm long, main lobe beaked, cucullate, shorter than the side lobes, whitish



1. Habit (1 cm = 4.8 cm) 2. spike (1 cm = 2.4 cm) 3. flower (1 cm = 29 cm), 4. Sterile bract (1 cm = 2 cm), 5. fertile bract (1 = 1 cm), 6. Comabract, 7. Calyx tube (1=1 cm), 8. Fruit (1=1 cm), 9. anther (1=0.6 cm), 10. Staminode side lobes (1=0.85 cm)

Fig. 1. ***Curcuma kshonapatra***

with pinkish tinge on the tip, 1.6 x 1.5 cm; side lobes oblong, 1.1-1. 3 cm x 0.8 - 0.9 cm in size, rounded tip, staminodes white, side lobes unusually very large and exerted and obovate, rounded, 3.0-3.5 cm long and 0.8-1 cm. wide; lip whitish, three lobed, mid lobe smaller than the side lobes in length and deeply emarginate, 1.7 x 1.5 cm in size, white with orange yellow median, stamen 1, anther basifixed, oblique, two celled, whitish, glabrous, 0.3 cm long, spurs 0.2 cm long, diverging, white, glabrous; ovary hairy, purple tinged, up to 4 mm long; stigma not exerted; fruit a capsule with persistent calyx, 1 cm long, globose, containing dark seeds with white translucent arils.

Type: *K. Velayudhan V/03-48* (holo NHCP), India, Karnataka, Udupi, 10 m MSL, 18.05.2003, (NHCP15288).

Live collections IC329331 and IC397735 (collector's numbers VBB/01-74 and V/03-47-B respectively) were conserved in the field genebank at NBPGR Regional Station, Thrissur.

Distribution: Udupi Town, Udupi district, Karnataka, India.

Etymology: The specific epithet 'kshonapatra' is based on purple-red tint along the midrib region of leaf.

Notes: Only 3 plants were noticed having similar features and hence in the first instance the author thought of ignoring such an entity. However, during September 2002 and May 2003 the author visited the site again to observe flowering in the population. Though the summer rains at Udupi was scanty in 2003, almost all the plants, numbering around 100, in the population (belonging to *C. albiflora* and the new species) were noticed in bloom. One plant with leaves having purple midrib could be noticed in bloom and this belonged to the present entity. Another site was a private compound with ornamental garden shaded by mango trees. Two plants were noticed there under disturbed condition owing to occasional weeding and also by burning the grass and litter falling from mango and jack fruit trees. This species could not be located near the coastal and adjoining sandy alluvial soils.

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Genetic Diversity Analysis of *Palas* [*Butea monosperma* (Lam.) Taub.] Flower Variants through Random Amplified Polymorphic DNA

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Palas [*Butea monosperma* (L.) Taub.], a multipurpose leguminous tree has numerous flower variants. The genetic diversity of the flower variants (golden yellow, yellow, mustard yellow, chrome yellow, white, and scarlet) and a morphological variant, s*wadi palas* was analyzed using RAPD marker. Thirty six RAPD primers were used to analyze their genetic diversity. Similarity coefficients of the *palas* variants ranged between 0.479 for yellow and *swadi palas* and 0.705 for mustard yellow and white variant. The cluster analysis grouped the *palas* variants into 2 major clusters, first comprising of golden yellow, yellow and chrome yellow variants and the second comprising of mustard yellow, white, scarlet and *swadi palas* variants. The unique polymorphic band of 687 bp was obtained in wild scarlet *palas* with OPS 02 primer. An attempt was made to develop more locus specific marker from this fragment. The newly designed locus specific primers amplified 687 bp fragment in chrome yellow, mustard yellow, white, scarlet and *swadi palas* variants. This marker will be helpful in identifying *palas* flower variants and their conservation and use in breeding programmes.

Key Words: *Butea monosperma*, Lac host plant, Molecular marker, *Palas*, RAPD

Introduction

Palas [*Butea monosperma* (L.) Taub], is an economically important multi-purpose tree species commonly found throughout India. It has numerous uses such as timber, fuel, fodder, tannin, gums and medicinal use especially in the folk medicine. In India, it is an important host tree for lac insect, *Kerria lacca* which produces commercial lac resin (Orwa *et al*., 2009). It has beautiful flowers adorning leafless canopies during early summer and hence commonly known as 'Flame of the Forest'. Traditionally, flowers have been used as astringent, aphrodisiac, diuretic, anthelmintic, in diarrhea, gynecological and various central nervous system disorders (Kumar and Malik, 2012). *Palas* has numerous variations in flower colour, number of leaflets, leaf shape and arrangement. *Palas* with scarlet coloured flowers is the most commonly available in nature and is supposed to be the wild type. The rare yellow and white flowering trees (Sanjappa, 1987), white flowers with yellowish central portion in Madhya Pradesh, India (Kamran, 1989), golden yellow, chrome yellow and mustard flower colour *palas* in Jharkhand, India (IINRG, Annual Report 2011-12) and rare variant with unifoliate leaves in Jharkhand (Kumar *et al*., 2006) have been reported in literature. *Swadi palas*, a morphological variant is found mainly in Jharkhand; the size of leaf and flower of which is larger than the normally occurring *palas*. Leaflets of *swadi palas* are acuminate in shape, and that of normal *palas* is obtuse. Flower colour of *swadi palas* is orange as compared to scarlet in normal *palas* (Lohot, 2011).

Considering the economical importance of this tree species and its medicinal values, the genetic diversity needs to be studied for genetic improvement and germplasm conservation in the wake of deforestation. Molecular markers have numerous merits over morphological markers as they are stable and detectable in all tissues despite any growth and development (Agarwal *et al*., 2008). Among different molecular methods available to study genetic diversity, RAPD (Random Amplified Polymorphic DNA) is the most simple, handy, inexpensive, rapid and powerful technique where sequence information of the target organism is lacking. RAPD is a PCR (Polymerase Chain Reaction) based marker and uses random decamer primers to bring out the polymorphism in the genotypes being compared (Williams *et al*., 1990; Welsh and McClelland, 1990). It has been widely used in the identification and genetic relationship analysis of a number of plant and animal species. Although the reproducibility of the RAPD marker is at stake in some cases, it can be converted

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to much more specific marker called SCAR (Sequence Characterized Amplified Region) marker. These SCAR markers generally reveal higher levels of polymorphism owing to higher annealing temperatures and longer primer sequence specificity (Kumla *et al*., 2012). The main advantage of SCARs is that they are quick, easy to use and require only low quantities of template DNA. In addition, SCARs have a high reproducibility and are locus-specific.

Genetic diversity of *palas* has been studied by previous workers from different agro-ecological zones using RAPD (Vaishali *et al*., 2008). DNA profiling of elite lac host *palas* trees with the help of RAPD was carried out (Vashishtha *et al*., 2014); genetic structure and diversity of natural populations of *palas* from different geographical regions were studied employing RAPD, ISSR (Inter Simple Sequence Repeats) and SRAP (Sequence Related Amplified Polymorphism) (Vashishtha *et al*., 2013). *Palas* flower colour variants were also analyzed by ISSR (Kandasamy *et al*., 2013). However, no attempt has been done so far in the development of SCAR in *palas* flower variants. Hence, RAPD marker was selected in this study to estimate the genetic diversity of *palas* flower variants and an attempt was also made to develop SCAR marker for *palas* variants from the polymorphic band obtained from the wild type *palas*.

Materials and Methods

The young leaves of *palas* flower variants were collected from different parts of Jharkhand during January 2013, transported in ice bags and stored at -80 °C until further use. The place of collection of *palas* variants and their geographical co-ordinates are given in Table 1. The study was conducted at Biotechnology laboratory of Lac Production Division of ICAR-IINRG, Ranchi from January to June 2013.

Genomic DNA was isolated from young *palas* leaves of the variants mentioned above using CTAB method (Doyle and Doyle, 1990) with minor modifications. One gram of leaf sample was weighed and crushed in liquid nitrogen. Finely ground leaf sample was transferred to a tube containing 10 ml CTAB. It was incubated at 55 °C for 1.5 hours and centrifuged at 12000 rpm for 5 min. Supernatant was taken in a 50 ml tube and equal volume of phenol: chloroform: isoamyl alcohol [25:24:1] was added. It was centrifuged at 12000 rpm for 15 min. The aqueous layer was transferred to another tube and 1/10th volume of 7.5 M ammonium acetate and 2-2.5 volume of ice cold absolute ethanol was added. It was incubated at -20 °C for an hour for better DNA precipitation. After that, it was centrifuged at 12000 rpm for 15 min. DNA pellet was washed with two changes of ice cold 70% ethanol. The residual ethanol was removed by air drying the DNA pellet. It was dissolved in TE buffer containing RNase (100 µg/ml). After suspending the DNA, it was incubated at 37 °C for 30 min. and stored at -20 °C. To purify the DNA, 1.5 volume of diluted binding buffer of HiPurA plant genomic DNA miniprep purification kit (cat. no. MB507, HiMedia, India) was added to 1 volume of DNA and mixed thoroughly. The mixture was loaded to mini preparation spin column and centrifuged at 8000 rpm for 2 min. After discarding flow through, the column was washed twice with wash solution. Then the genomic DNA was eluted with elution buffer in 1.5 ml tube. Purity and quantity of DNA was checked using Nanodrop 2000 spectrophotometer (Thermo scientific, USA).

RAPD Analysis

Forty random decamer primers were used for the amplification of genomic DNA by PCR. Primers used were of OPS and OPH series. The volume of the final reaction was of 25 µl. It was made up of 1X buffer,

Table 1. *Palas* variants used in the study and their place of collection

S.No.	Variant	Place of collection	Latitude	Longitude
1.	Golden yellow	Khakhikalan, Giridih, Jharkhand	$23^0$56’53.6”N	$86^0$01’48.3”E
2.	Yellow	Khakhikalan, Giridih, Jharkhand	$23^0$56’55.37”N	$86^0$01’49.07”E
3.	Mustard yellow	Chainpur, Giridih, Jharkhand	$24^0$00’41.59”N	$86^0$01’58.50”E
4.	Chrome yellow	Bahadurpur, Bokaro, Jharkhand	$23^0$39’38.44”N	$85^0$57’10.49”E
5.	White	IINRG Research farm, Ranchi, Jharkhand	$23^0$19’51.2”N	$85^0$22’06.3”E
6.	Scarlet	IINRG Research farm, Ranchi, Jharkhand	$23^0$19’51.2”N	$85^0$22’06.3”E
7.	*Swadi palas*	Putadag, Angara, Jharkhand	$23^0$25’.746”N	$85^0$22’.478”E

4 mM $MgCl_2$, 0.1 mM dNTPs, 10 pico mole primers, 1.25 U *Taq* DNA polymerase (MBI Fermentas, USA) and 10 ng of template DNA. Amplification was carried out in labcycler (Sensoquest, Germany) with an initial denaturation period of 2 min at 94 °C, followed by 40 cycles of 30 sec at 94 °C, 30 sec annealing at 35 °C, 2 min extension at 72 °C and a final extension step of 10 min at 72 °C. The PCR products were run on 1% agarose gel and DNA bands were visualized by ethidium bromide staining and saved in Gene Genius Bioimaging system (Syngene, UK). Each PCR was repeated twice to confirm reproducibility.

Scoring of RAPD Data and Construction of Dendrogram

Only the clear, unambiguous and reproducible bands present across the DNA samples were used for scoring. With reference to molecular weight marker, at particular band size, the presence of bands was scored '1'; while the absence of bands or very faint bands was scored '0'. Information content of the RAPD primers was estimated through Diversity Index (DI), Marker Index (MI) and Resolving Power (RP). Marker Index was calculated using the following formula MI = Diversity index (DI) X Effective Multiplex Ratio (EMR). DI of a primer is defined as $1 - \Sigma P_i^2$, where P_i is the band frequency of the *i*th allele. EMR is the product of number of polymorphic bands (*i.e.* absence of band at least in one genotype at a particular locus) per primer and the fraction of polymorphic bands (Milbourne *et al*., 1997). Resolving power was calculated as per the following formula $Rp = \sum I_b$. Band informativeness, $I_b = 1-[2 X |0.5-p|]$, where p is the proportion of the seven genotypes containing the band (Prevost and Wilkinson, 1999). The binary data were analyzed using NTSYSpc software version 2.02i. The similarity matrix was constructed based on Jaccard's coefficient. Clustering was done by UPGMA using SAHN module and the dendrogram was created subsequently.

Cloning of Polymorphic RAPD Locus of Wild Scarlet Palas

Unique and polymorphic band of around 700 bp was obtained from the wild scarlet *palas*. It was eluted from the gel using QIAquick gel extraction kit (Qiagen, Germany) following manufacturer's instructions. The eluted fragment was ligated with pGEM Teasy vector (Promega, USA). *E. coli* DH5α cells were transformed with the ligation mix and the recombinant clones were selected on the LB agar plate containing ampicillin, X-gal and IPTG. Plasmids were isolated from the white colonies and the size of the inserts was confirmed by PCR and restriction digestion with *EcoRI* enzyme. The plasmids having correct size insert were sequenced using T7 and SP6 primers at the sequencing facility of Xcelris Labs Ltd. Ahmedabad, India. The sequence was analyzed using Geneious software ver. 6.1.6. BLAST analysis was also carried out to find homologues for the sequence obtained (http://blast.ncbi.nlm.nih.gov/Blast).

Designing of Locus Specific Primers

The sequence obtained was used to design sequence characterized amplifiable region (SCAR) primers. The primer sequences are given below:

Sc.F2: 5' CTG ACT GTC ATA ATA AGT TCT A 3'

Sc.R2: 5' CCT CTG ACT GGG CAA AGC 3'

The primers were used to amplify all *palas* variants to check their specificity. The volume of the final reaction was of 25 µl. It was made up of 1X buffer, 2 mM $MgCl_2$, 0.1 mM dNTPs, 10 pico mole primers, 1.25 U *Taq* DNA polymerase and 25 ng of template DNA. Amplification was carried out in Sensoquest thermal cycler with an initial denaturation period of 2 min at 94 °C, followed by 35 cycles of 30 sec at 94 °C, 30 sec annealing at 60 °C, 2 min extension at 72 °C and a final extension step of 10 min at 72 °C. The PCR products were run on 1% agarose gel and DNA bands were visualized by ethidium bromide staining and saved in Gene Genius Bioimaging system (Syngene, UK).

Results and Discussion

The present investigation was carried out to study the genetic diversity in *palas* variants using RAPD markers. Genomic DNA was isolated from six flower variants and a morphological variant, *swadi palas*. The purity index of genomic DNA isolated from the *palas* variants ranged from 1.8 to 2.0 showing the DNAs obtained were of good quality, without any contamination.

A total of 40 arbitrary decamer primers (OPS and OPH series) were used for initial screening. Out of 40 primers used, 36 could generate good PCR profile and reproducible results. Vaishali *et al.* (2008) used 30 RAPD primers for *B. monosperma*, of which only 12 generated reproducible results. The selected 36 primers were used for RAPD analysis of 7 *palas* flower variants. The present RAPD analysis generated 450 bands across all 7 variants. A representative picture for RAPD profiling

of *palas* variants with OPS 10 primer is shown in Figure 1.

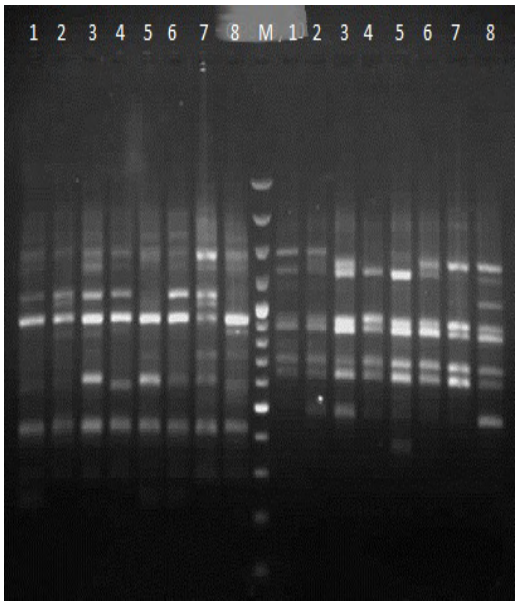

Fig. 1. RAPD profile of *palas* variants using OPS 10 primer. Lane M- 100 bp ladder, 1-golden yellow, 2-yellow, 3-mustard yellow, 4-chrome yellow, 5-white, 6-scarlet flower colour variants and 7-s*wadi palas* variant

It has been reported that the ability to resolve genetic variation may be more directly related to the degree of polymorphism detected by the marker system. Primers OPH 01, OPS 05, and OPS 16 showed 100% polymorphism while least polymorphism was shown by OPS 03 (43.75%). The average polymorphic band percentage was found to be 70.44%. However, a lesser level of polymorphism (66.3%) was obtained for elite lac host *palas* trees (Vasishtha *et al*., 2014) and higher level of polymorphism (86%) for *palas* collected from different agro-ecological zones of India (Vaishali *et al*., 2008). Since the amplification depends upon the sequence of RAPD primers, coverage of genome by the primers and genetic nature of genotypes used, the rate of polymorphism tend to vary. A wide range of polymorphism occurs in the tree crops from as low as 10.48% in *Pongamia pinnata* (L), Panigrahi (Kesari *et al*., 2010) to as high as 99.39% in *Grevillea* species with RAPD markers (Pharmawati *et al*., 2004). Average number of bands per primer was found to be 12.5, which was higher than the RAPD analysis of *B. monosperma* by Vaishali *et al*. (2008) and Vasishtha *et al*. (2013). On an average each primer produced nine polymorphic bands, which was also more than the one (5.6) reported by Vasishtha *et al*. (2013) for RAPD analysis for *palas* trees. A maximum of 21 scorable bands were recorded with primer OPH 12, whereas OPS 06 showed a minimum of five scorable bands.

An efficient marker system must be capable of distinguishing different genotypes. Resolving Power (RP) depends on the distribution of the alleles within genotypes and estimates the capacity of discrimination of each primer. The resolving power of primers used in the study ranged from 1.42 to 8.57. OPS 05 and OPS 15 were the most informative primers having the highest RP of 8.57 and OPS 01 showed the least RP of 1.42. A very high range of RP from 3.85 to 19.14 with an average of 10.46 per primer was reported for 14 *Thymus* accessions (Yousefi *et al*., 2015). OPS 15 primer was able to differentiate 6 *palas* flower variants.

The Diversity Index (DI) value of the primers ranged from 0.69 to 0.99. The Marker Index (MI), which is the product of DI and EMR, was used to evaluate the overall utility of each marker system. The parameter, MI was first used by Milbourne *et al.* (1997) to compare different molecular markers in genetic relationship studies and the MI was defined as a general measure of efficiency of the primers in detecting polymorphism. MI was calculated for all the RAPD primers in this study and found to vary from 1.25 (OPS 06) to 13.75 (OPS 05). However, MI varied between 2.9 and 15.85 with an average of seven for ISSR primers used in the genetic diversity analysis of *palas* flower colour variants (Kandasamy *et al*., 2013).

Similarity matrix was constructed for *palas* variants using Jaccard's co-efficient and tabulated in Table 2. The similarity co-efficient values ranged from 0.479 (between yellow and *swadi palas*) to 0.705 (white and mustard yellow). Jaccard similarity matrix based on RAPD binary data was used to cluster the *palas* variants following Unweighted Pair Group with Arithmetic Mean (UPGMA) method. The dendrogram (Fig. 2) revealed that *palas* variants could be clustered into two major groups — first group consisted of three variants: golden yellow, yellow and chrome yellow colour variants of the *palas* while second group consisted of four variants: mustard yellow, white, scarlet colour variants and *swadi palas*. This result is comparable with the ISSR studies of flower colour variants of *palas* (Kandasamy *et al*., 2013), wherein golden yellow and yellow variants were in one cluster and rest of the variants were in another cluster.

Table 2. Similarity coefficients of *palas* variants

Variant	Golden yellow	Yellow	Mustard yellow	Chrome yellow	White	Scarlet	*Swadi Palas*
Golden yellow	1.000						
Yellow	0.659	1.000					
Mustard yellow	0.606	0.540	1.000				
Chrome yellow	0.656	0.633	0.613	1.000			
White	0.587	0.538	0.705	0.602	1.000		
Scarlet	0.597	0.541	0.671	0.629	0.683	1.000	
Swadi Palas	0.559	0.479	0.675	0.565	0.673	0.661	1.000

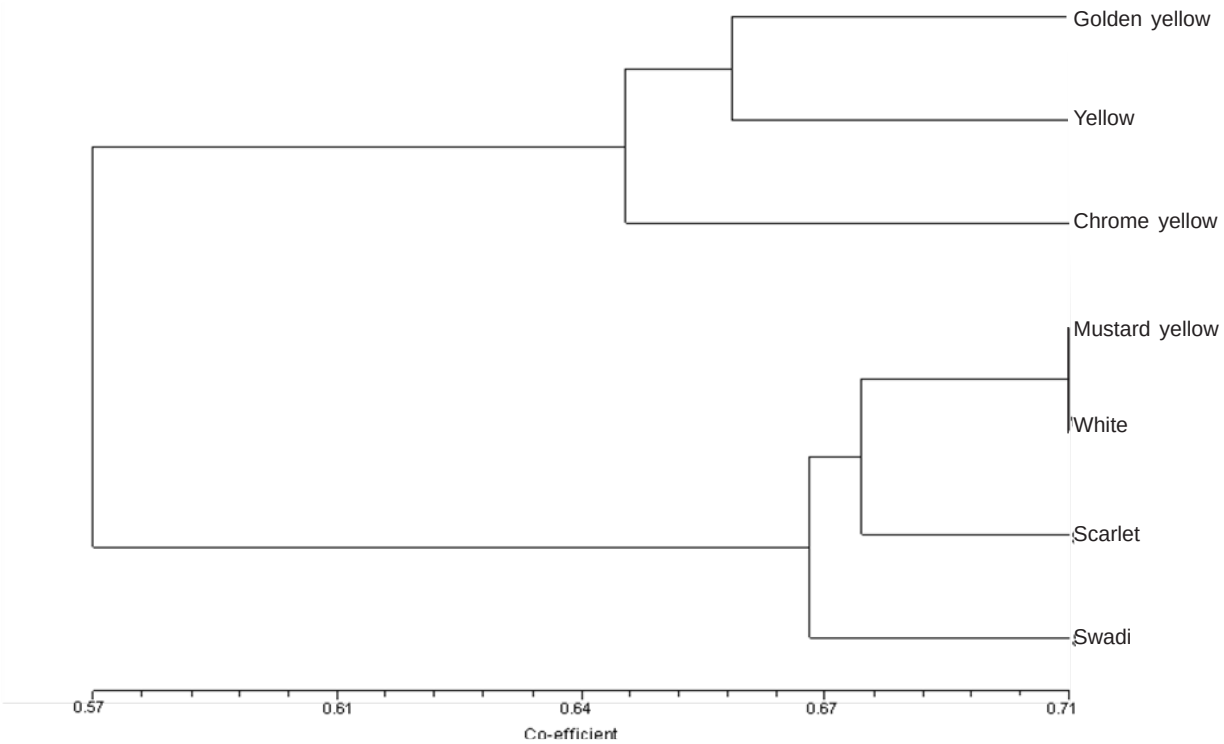

Fig. 2. Dendrogram of *palas* variants based on RAPD data

The primer, OPS 02 generated a unique, locus specific polymorphic fragment of 687 bp size with wild type scarlet *palas* (Fig. 3). This fragment was cloned and sequenced. The sequence obtained was of 687 bp and is given below. The OPS 02 primer sequence is shown in lower case.

c c t c t g a c t g T C A T A A T A A G T T C T A G T T A T G G G C T A A T G A T G C C T A C T T T T A G C T T A G T C
A T A A T T T G T T T G T T G T G C C C A A T T A G G G G C T T T T T A G T T T T G T A T T T A A A C A C C A A
C T G T A A T A C T T C A A G T G T G T G T A A A A G T T G T G T G T G A A A A A C T A A A A T A C A T T A A
A T G A G T T T G T G C T C T T T T C T C C T T T T T T G T A T G T A T G G T T G T C A T T T T C C T T A A A G C C
T T T T T G C T A G T T T A G T C C A A T A G C T T T G T T G C T G A G T C T A A T A G T T T T C C A T C A A G
T C T A A T A A C T T T T T C A A T A G T T T A A A A A C C T G T T C T A T T T T T C A A C A G A T A T C T A A A
A G C A G A A A A G T T A G A G A A G T G A G G A A T A C T T A A T A A C A A T G G C C T G G G C C T G T A
G G C T G G G G G G A G G T T C A T C T C C C T A T C A A T A A A G A G C A T T C C T T A T C A T G T A T T T
T T A A T G G C T A T T A C A G C A G A C A G T G G C C A A A T G T T A T G C C A A A A A A T G A G G A G T
G T T G G A A A T T G A G A T T T T T C C A A C T A T C A T A A T A A A A T A T T T T A A T G T C T C T G T A G
T T T T C T A C T T G C T T G T A A G T T T G G T T A A A G G T T G T T T A T G T T T A G A A A A A G C T T G G
TTATAGTTGTGGGCTAATGATGCTTACTTTTAGCTTAGCCATAATTTGTTATTTGC TTTGCCcagtcagagg

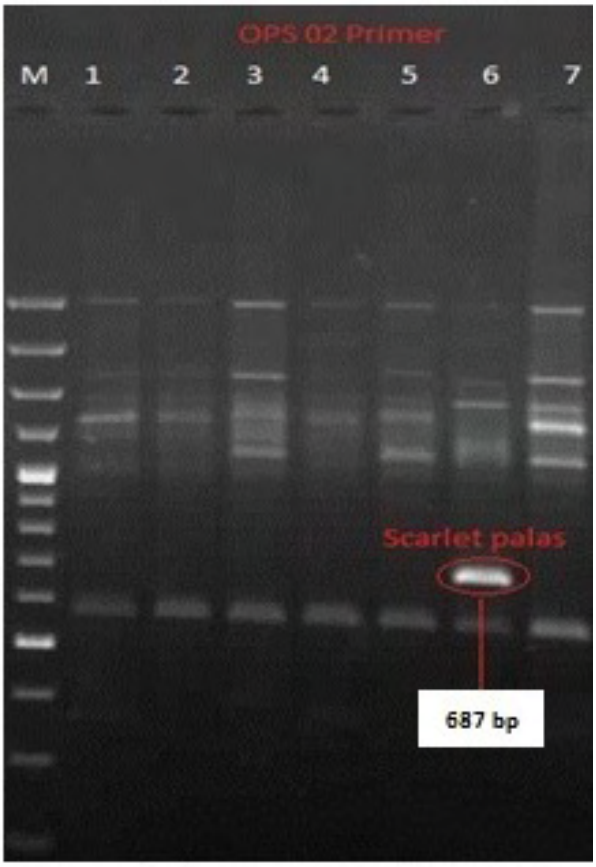

Fig. 3. RAPD profile of palas variants using OPS 02 primer. Lane M- 100 bp ladder, 1-golden yellow, 2-yellow, 3-mustard yellow, 4-chrome yellow, 5-white, 6-scarlet colour variants and 7-swadi palas variant

When the sequence was used for homology BLAST search in NCBI GenBank, it revealed that 120 bp of the query sequence (from 549 to 669 nucleotides) shared 80 % identity with *Glycine max* uncharacterized (LOC100790248) transcript variant X5, ncRNA with E value of 2e-13. Hence it could be considered as some uncharacterized region form the *palas* genome.

Based on the sequence of 687 bp, longer locus specific primers (Sc.F2 and Sc.R2) were designed. The newly designed primers were tested against *palas* flower variants. The primers amplified all *palas* variants except golden yellow and yellow flower colour variants (Fig. 4). Although it was anticipated that the primers have to amplify specifically scarlet *palas,* the primers amplified chrome yellow, mustard yellow, white, scarlet and *swadi palas*. However, it did not amplify golden yellow and yellow, which is in a different cluster of the dendrogram. In RAPD, primers compete for binding site and might have missed a particular locus of 687 bp in other *palas* variants as obtained in scarlet *palas* with OPS 02 primer. However, in locus specific primers there is no competition for the primers and could have amplified the same locus in other variants including mustard yellow, chrome yellow, white and *swadi palas* besides scarlet *palas*. In natural conditions, mostly yellow *palas* and golden yellow *palas* trees are found in nearby locations. The locus specific primer set (Sc. F2XSc.R2) could be used for differentiating yellow and golden yellow *palas* variants from other flower variants even before flowering, which will be highly useful in selection of trees for plantation or for medicinal uses.

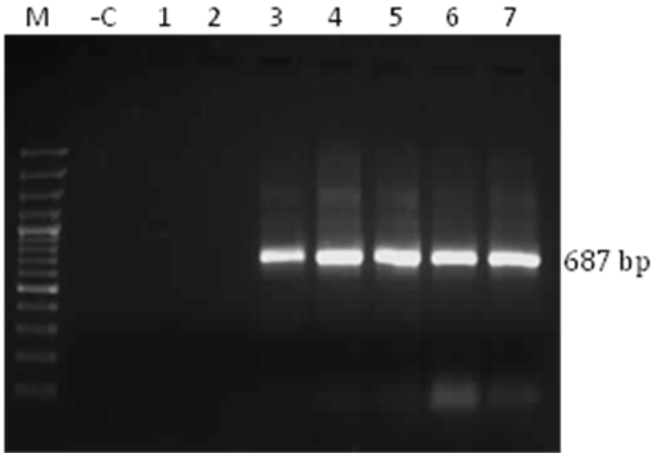

Fig. 4. PCR profile of *palas* variants using locus specific primers Sc.F2XSc.R2. Lane M- 100 bp ladder, -C-negative control, 1-golden yellow, 2-yellow, 3-mustard yellow, 4-chrome yellow, 5-white, 6-scarlet colour variants and 7-s*wadi palas* variant

Palas trees are under heavy exploitation due to its medicinal values and continuous threat of deforestation. The genetic base may be eroded because of these reasons and conservation of its genetic base is very essential. RAPD markers used in the study were highly efficient and suitable for diversity analysis of *palas* flower variants generating sufficient polymorphic data and to identify flower variants for future genetic improvement of this tree. Similarly, the SCAR marker which is amplifying non-yellow and golden yellow flower variants will also be helpful in conservation strategies and in genetic improvement programmes. However, the specific SCAR makers for flower variants may be developed by screening more number of RAPD primers, which would be much more informative and useful in the future breeding programmes.

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SHORT COMMUNICATION

Analysis of Genetic Diversity in *Alpinia galanga* using ISSR Markers

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Genetic diversity analysis was carried out in *Alpinia galanga* accessions collected from Kerala state of India using Inter Simple Sequence Repeats (ISSR) markers. A total of 60 bands were generated from 18 accessions of *A. galanga* using 11 ISSR primers. Of these 24 (40%) were polymorphic and remaining 36 (60%) were monomorphic. Total Nei's genetic diversity among the three populations was estimated as h= 0.1735 $\pm$ 0.013 and I= 0.2470 $\pm$ 0.078. Nei's estimator of population substructure (G_{ST}) indicated a fairly low level of population differentiation (G_{ST} = 0.2139). Based on this study we propose a conservation strategy to this species.

Key Words: *Alpinia galanga*, Genetic differentiation, Genetic diversity, Greater galangal, ISSR

Alpinia galanga (Linn.) Willd. (family Zingiberaceae) is an important medicinal-cum-aromatic plant distributed in India, Indo-China, Philippines and Borneo (Sabu, 2006). It is commonly called as Greater galangal or *Valia-aratha*. It is a clonally propagated species and is sparsely represented in western ghats of India (Sabu, 2006). The aromatic rhizome of *A. galanga* is extensively used in traditional medicines like Ayurvedic, Unani, Chinese, and Thai folk systems to cure diseases such as bronchitis, diabetes, heart diseases and kidney disorders. It is also effective to alleviate pain specifically head-ache, chest pain and rheumatic pain. The rhizome extract has the potential to stimulate digestion, purify blood and to improve voice (Sabu, 2006). It has also been listed among the promising cancer preventing dietary supplements (Ohigashi, 2002). Raw rhizome is an essential ingredient in many Indonesian and Malaysian dishes for its ginger-like flavour.

Genetic study of clonal plant species provides reliable information on their population dynamics and detailed demographic data (Kareem *et al*., 2012; Rajasekharan and Kareem, 2010). To the best of our knowledge, genetic characterization of *A. galanga* is insufficient to propose a suitable conservation strategy (Saritnum and Sruamsiri, 2003). The present study focussed on the genetic diversity analysis in *A. galanga* accessions collected across Kerala state of India using Inter Simple Sequence Repeats (ISSR) markers.

ISSR amplifies inter-microsatellite sequences at multiple loci throughout the genome (Rajasekharan and Kareem, 2010). These markers offer a great potential to determine intra- and inter-genomic diversity compared with other arbitrary primers, since these reveal variations within unique regions of the genome at multiple loci simultaneously. ISSRs have become popular and effective markers for measuring genetic diversity in clonal plants as-well-as in medicinal plants (Kareem *et al*., 2011, 2012; Mohan *et al*., 2013; Rajasekharan and Kareem, 2010, 2015).

A survey was conducted to collect *A. galanga* populations in Kerala state of South India. A total of eighteen accessions were collected from different locations along with their geographical coordinates. From the study, three populations were identified *viz*., Trivandrum zone, Cochin zone and Calicut zone. The altitudes of the collection sites were ranged from 17 m to 204 m.

Genomic DNA was extracted from fresh leaves (0.5g) of *A. galanga* using modified CTAB method (Sane *et al*., 2012). ISSR-PCR reaction was performed in a volume of 10 µl containing 50 ng template DNA, 0.5mM dNTPs (Chromous Biotech, Bangalore, India), 0.15U *Taq* DNA

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polymerase (Chromous Biotech), 0.5µM ISSR primers from University of British Columbia (The Michael Smith Laboratories, University of British Columbia, primer set # 9, Vancouver, BC, Canada) and 1X PCR buffer (10mM Tris HCl pH 8.3, 50mM KCl, 3mM $MgCl_2$) (Merck). A total of eleven reproducible primers were selected from 100 ISSR primers for the present study based on the consistent banding pattern (Table 1). The reactions were carried out in a DNA thermocycler (Eppendorf mastercycler gradient, Germany) with an initial denaturation step of 94°C for 4 min, followed by 34 cycles at 94°C for 1 min, 45 sec at the specific annealing temperature of each primer (44 to 56°C) and 72°C for 1 min and a final extension at 72°C for 8 min and a hold temperature of 4°C at the end. After amplification, the reaction products were subjected to electrophoresis in 1.5% agarose gels in 1x TAE buffer stained with 5µg ml^{-1} ethidium bromide and photographed under UV light with the help of a gel documentation system (Syngene). 1Kb molecular ladder was used as marker to know the size of the fragments.

Amplified products, which were reproducible and consistent in performance, were scored for band presence (1) or absence (0) and a binary qualitative data matrix was constructed. Percentage of polymorphic bands (PPB) was calculated by dividing the number of polymorphic bands by the total number of bands surveyed. The binary matrix was used to determine the genetic diversity, genetic differentiation and gene flow using the software PopGene version 1.31 (Yeh *et al.*, 1999). Genetic diversity within and among populations were measured by PPB, effective number of alleles (ne), observed number of alleles (na), Nei's (1973) gene diversity (*h*) and Shannon's information index (*I*). At the species wide level, total genetic diversity (H_T) and genetic diversity within populations (H_S) were calculated. To estimate the genetic divergence among populations, we also calculated the relative magnitude of genetic differentiation among populations ($G_{ST} = (H_T - H_S)/H_T$). Corresponding estimates of gene flow (*N*m), *i.e.* the average per generation number of migrants exchanged among populations, was estimated using the formula: $Nm = 0.5(1 - G_{ST})/G_{ST}$.

In addition, an analysis of molecular variance (AMOVA) was performed to calculate the partitioning of genetic variance among and within population using GenAlEx ver. 6.41 (Peakall and Smouse, 2006). The permutation number for significance testing was set to 999 for all the analysis. To explore the genetic relationships among all populations, a UPGMA (Unweighted Pair-Group Method using Arithmetic average) dendrogram was constructed based on the matrix of Nei's genetic distance by the program TFPGA, version1.3. Given that the above estimation of allele frequencies from dominant markers requires the assumption of Hardy–Weinberg equilibrium.

Table 1. Details of ISSR primers used for PCR amplification of *A. galanga**

S.No.	Primer No	Primer sequence	Total number of bands obtained	Percentage of Polymorphic bands
1	811	$(GA)_8C$	3	66.6
2	814	$(CT)_8A$	5	60
3	827	$(TC)_8A$	4	50
4	834	$(AG)_8YT$	5	40
5	844	$(CT)_8RC$	3	33.3
6	855	$(AC)_8YT$	5	40
7	857	$(AC)_8YG$	5	40
8	866	$(CTC)_6$	6	33.3
9	873	$(GACA)_4$	3	33.3
10	887	DVD $(TC)_7$	10	30
11	902	CTC $(GT)_8$	5	40
Total / Av.			60	41.66

*D = (A, G, T); R = (A, G); Y = (C, T).

A total of 60 bands were generated from 18 accessions of *A. galanga* by using 11 ISSR primers (Fig. 1). Of these 24 (40%) were polymorphic and remaining 36 (60%) were monomorphic. Number of bands varied between 3-10 and average 5 bands per primer was observed (Table 1). Total Nei's genetic diversity among the three populations was estimated as $h = 0.1735 \pm 0.013$ and $I = 0.2470 \pm 0.078$ (Table 2). South Kerala population exhibited more genetic diversity (PPB= 40%; $h = 0.1699 \pm 0.071$; $I = 0.2437 \pm 0.10$) than the other two populations. Average genetic variation within population was observed as $h = 0.1364 \pm 0.009$. The observed and effective number of alleles across the populations were $na = 1.4 \pm 0.11$ and $ne = 1.32 \pm 0.11$ respectively. The current study reveals a relatively a low level genetic diversity exist within and among populations of *A. galanga*. The low genetic diversity may be due to high rhizome distribution as a clonal propagule.

Across the three populations of *A. galanga* surveyed for ISSR variation, Nei's estimator of population substructure (G_{ST}) indicated a fairly low level of population differentiation ($G_{ST} = 0.2139$) (Table 2). These G_{ST} values translated into correspondingly

Table 2. Analysis of genetic diversity in *A. galanga* population detected by ISSR*

Population	h	I	PPB	G_{ST}	Nm
Trivandrum zone	0.1699 ± 0.071	0.2437 ± 0.10	40%		
Cochin zone	0.0923 ±0.091	0.1328 ± 0.12	21.67%		
Calicut zone	0.1470 ± 0.124	0.2100 ± 0.17	33.33%		
Mean	0.1364 ± 0.009	0.1955	31.66%		
Species level	0.1735 ± 0.013	0.2470 ± 0.078	40%	0.2139	1.8375

**h*, Nei's (1973) diversity index; *I*, Shannon's information index; PPB, percentage of polymorphic loci; G_{ST}, genetic differentiation between populations (Nei's); *N*m, estimated

high levels of gene flow (*Nm*), with 1.8375 migrants exchanged between populations (on average) each generation. The AMOVA also revealed that all of the genetic variation exists within the populations rather than among the populations. The dendrogram obtained using the UPGMA algorithm based on Nei's genetic distance is presented in Fig. 2. The analysis showed that populations of Trivandrum zone and Calicut zone exhibited more closeness whereas those of Cochin zone was little diverged from the two.

The information on genetic diversity and relationship within and among crop species is essential for the efficient utilization of plant genetic resource collections (Rajasekharan and Kareem, 2010). Our study is concerned with the evaluation of the genetic diversity and relationship of *A. galanga*. It could be explained that *A. galanga* has been concurrently moved across vast geographic locations within India; therefore, genetic differentiation of *A. galanga* has been affected. Genetic improvement of *A. galanga* should be based on molecular variation as well as morphological differences. Our investigation of genetic diversity by ISSR markers clearly showed that there is low genetic variation within and among *A. galanga* populations in Kerala. Similar result was observed in some clonally propagated species, such as *Alternanthera philoxeroides* (Wang *et al*., 2005). In general, clonally propagating plant species exhibit low genetic variation within and among population. It is widely accepted that population genetic variation is influenced by factors, such as historical events, breeding system, genetic drift and natural selection. Relatively low genetic diversity in *A. galanga* may be related to its introduction or cultivation history. It is thought that, from the present study, the source material for the Kerala population might have come from single propagule or from closely related propagules. *A. galanga* usually propagates through its rhizome which makes genetically uniform population.

The population genetic structure of a species is affected by a number of evolutionary factors including mating system, gene flow, extent of population diversity as well as natural selection (Hamrick and Godt, 1990). The clonal propagation theoretically has similar effects for population genetic structure as strict selfing (inbreeding). Inbreeding mode usually reduces gene exchange (gene flow) both between different individuals

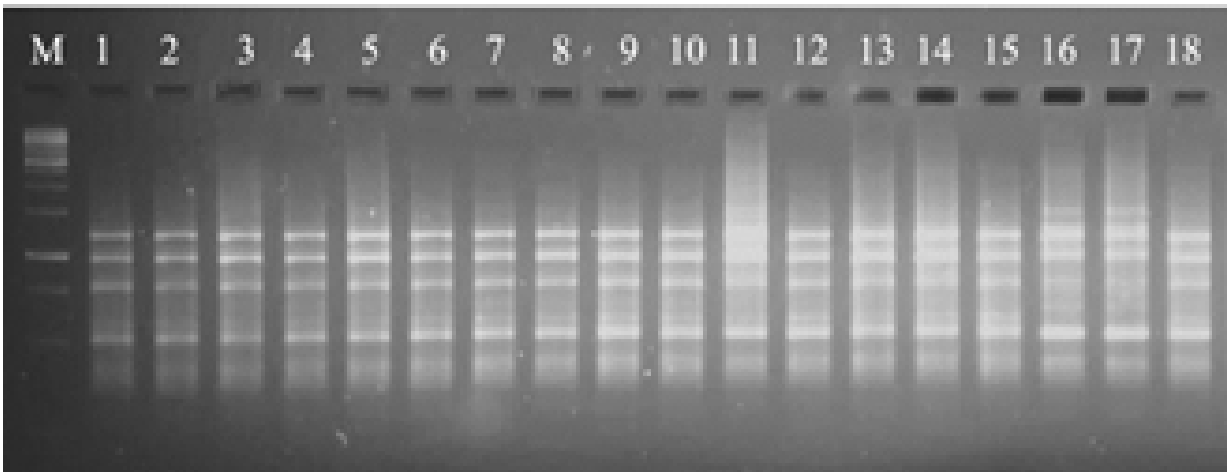

Fig 1. ISSR profile of 18 accessions of *A. galanga* Lane M- DNA marker, Lanes 1 to 18 ISSR profiles of individual accessions of *A. galanga*

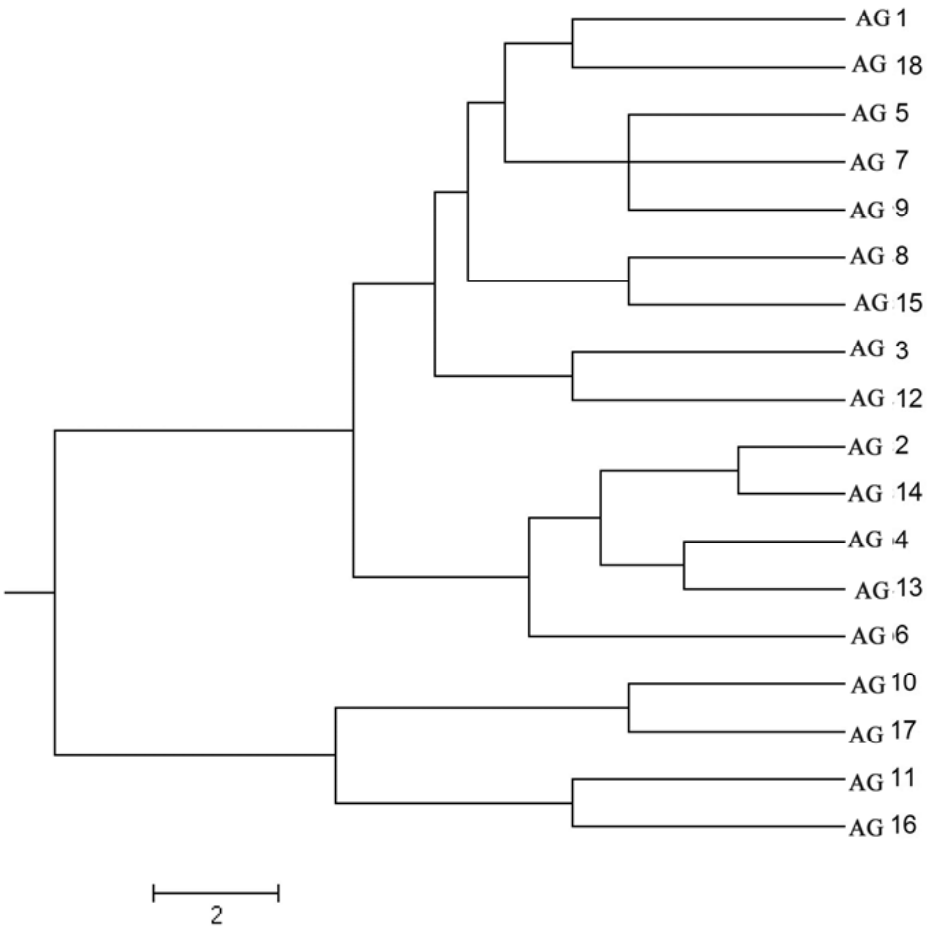

Fig. 2. UPGMA dendrogram showing genetic relationship between 18 accessions of *A. golanga* based on 60 ISSR markers

and populations, leading to significant differentiation between populations. The value of G_{ST} observed in the present study was much lower than the average G_{ST} for selfing species (0.51 (Hamrick and Godt, 1990)). This is thought to be due to the reproductive mode of *A. galanga*.

Could the cultivation practices result in the homogeneity or decrease of genetic diversity after several decades of cultivation? This question becomes more important while wild gene pools of this species decreased rapidly and lack of good cultivars. Clonal reproduction does not involve recombination and, therefore, yields offspring that are genetically identical to each other and to the mother plant. The extent of genetic diversity of *A. galanga* in cultivated populations in present studies was lower as in the case of any clonally propagated plant. Genetic differentiation and gene flow are important index to evaluate the population genetic structure of a species. Gene flow, the movement of gene within and between populations, is negatively correlated with genetic differentiation, but is very important for population transfer and plant evolution. Conservation of wild plants has a major focus on preserving viability of existing populations through *in situ* programme. Since realization of importance of *ex situ* collections for conservation *in situ*, limitations of their utility became evident.

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SHORT COMMUNICATION

Relative Productivity of Important Pasture Grasses and Their Genotypes in Arid Zone of India

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Three separate field experiments on selection of high yielding genotypes of *Cenchrus ciliaris, Cenchrus setigerus* and *Lasiurus sindicus* were conducted at Agricultural Research Station, Keshwana, Jalore, Rajasthan (India) for two years (2010-11 and 2011-12). A wide range of variation has been observed for forage yield among pasture species and their genotypes. Green forage yield of *C. ciliaris* ranged between 256.63 and 345.19 q/ha with an average of 290 q/ha. In *C. setigerus*, forage yield ranged between 210.07 and 292.39 q/ha with an average of 264.84 q/ha. In case of *Lasiurus sindicus,* forage yield ranged between 185.31 and 222.95 q/ha with an average of 204.95 q/ha. The overall pasture productivity of *C. ciliaris* was found 55.32 and 9.50% higher over *L. sindicus* and *C. setigerus*, respectively. Therefore, high yielding genotypes of *C. ciliaris* and *C. setigerus* may be preferred for pasture development programme in district Jalore (Rajasthan).

Key Words: *Cenchrus ciliaris, C. setigerus,* Forage yield, Genotypes, *Lasiurus sindicus*

The adequate supply of nutritious forage and feed is the key for successful animal husbandry, which is providing livelihood support and nutritional security to rural inhabitants (Sharma, 2013). Pasture grasses are most important and cheapest source of perennial forage for grazing animals under rainfed situation (Yadav, 1995). Rootstock of perennial grasses sprouts after onset of monsoon and physiologically matures with the withdrawal of monsoon. In India 12 million hectare is said to be under permanent pastures and grazing lands but biomass production from these lands is very meagre (Singh, 1989). *Cenchrus ciliaris, Cenchrus setigerus* and *Lasiurus sindicus* are drought tolerant and high yielding perennial pasture grasses with wider adaptability in arid zone (Singh and Singh, 1995). Saxena and Singh (1976) reported that sand dunes and sandy undulated aggraded older alluvial and inter-dune plains and sandy undulating buried pediments in the form of dunes and hummocks of district Bikaner may be developed by trees, shrubs and grasses like *C. ciliaris, C. setigerus* and *L. sindicus.* These grasses are found as sole pasture and with woody perennials under silvipastoral system. Forage of the grasses is highly palatable, digestible and nutritious to all kind of animals. In arid zone, the production and productivity of these grasses is highly erratic and varies year after year with the amount and distribution of rainfall. Considerable efforts have been made at national and state level to enhance pasture production by adopting suitable grasses, their high yielding cultivars and technological interventions. The present paper deals with the identification of suitable pasture grasses for district Jalore (Rajasthan).

Three separate experiments on evaluation of high yielding genotypes of *Cenchrus ciliaris, Cenchrus setigerus* and *Lasiurus sindicus* under All India Coordinated Research Project on Forage Crops were conducted at Agricultural Research Station, Keshwana, Jalore, Rajasthan (India). The experimental site is situated at latitude 25º23.115'N, longitude 72º 30.726'E, elevation 149.9 msl and has a tropical arid climate with mean annual rainfall of 421 mm. Soil at the site was clay loam slightly saline in reaction (pH 8.7) and low in organic carbon (0.23 %). In first trial, six genotypes of *Cenchrus ciliaris* were sown on July 11, 2008 in a randomised block design with four replications. In second trial, eight genotypes of *Cenchrus setigerus* and in third trial seven genotypes of *Lasiurus sindicus* were sown on July 8, 2010 in a randomised block design with three replications. One genotype of *L. sindicus* could not get germination. Plot size was kept as 2.5m

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Table 1. Forage yield and plant height of different pasture grasses (Average of two years)

S. No.	*Cenchrus setigerus*			*Cenchrus ciliaris*			*Lasiurus sindicus*		
	Genotypes	GFY (q/ha)	Plant height (cm)	Genotypes	GFY (q/ha)	Plant height (cm)	Genotypes	GFY (q/ha)	Plant height (cm)
1	VTCS-1	274.73	110.91	CE-08-1	293.13	133.75	IVTS-1	185.31	89.67
2	VTCS-2	210.07	101.58	CE-08-2	256.63	118.75	IVTS-2	222.95	100.00
3	VTCS-3	264.33	102.68	CE-08-3	293.16	128.75	IVTS-3	214.25	95.33
4	VTCS-4	246.21	99.22	CE-08-4	345.19	130.00	IVTS-5	197.96	75.00
5	VTCS-5	291.87	108.44	CE-08-5	277.51	118.13	IVTS-6	209.89	91.67
6	VTCS-6	259.93	108.12	CE-08-6	274.38	117.50	IVTS-7	199.34	88.00
7	VTCS-7	292.39	107.44	Mean	290.00	124.48	Mean	204.95	89.95
8	VTCS-8	279.20	97.78	-	-	-	-	-	-
	Mean	264.84	104.52	-	-	-	-	-	-

GFY= Green forage yield

x 4.0 m accommodating five rows at 50 cm spacing. The experiment was managed with standard package of practices under rainfed situation. Data collected on green forage yield and plant height was analysed using standard analysis of variance (ANOVA) through Excel software of Microsoft Office.

Results revealed a wide range of variability among genotypes for green forage yield and plant height in all three pasture species. In *Cenchrus ciliaris*, green forage yield ranged between 256.63 (CE-08-2) and 345.19 q/ha (CE-08-4) with an average of 290 q/ha. Plant height of genotypes ranged between 117.50 (CE-08-6) and 133.75 cm (CE-08-1) with an average of 124.48 cm. In *Cenchrus setigerus*, green forage yield ranged between 210.07 (VTCS-2) and 292.39 q/ha (VTCS-7) with an average of 264.84 q/ha; and plant height of genotypes ranged between 97.78 (VTCS-8) and 110.91 cm (VTCS-1) with an average of 104.52 cm. The green forage yield of different genotypes of *Lasiurus sindicus* ranged between 185.31 (IVTS-1) and 222.95 q/ha (IVTS-2) with an average of 204.95 q/ha; and plant height of genotypes ranged between 75 (IVTS-5) and 100 cm (IVTS-2) with an average of 89.95 cm.

The comparative performance of pasture grasses revealed that maximum green forage yield of 290 q/ha was produced by *C. ciliaris* followed by *C. setigerus* and *L. sindicus* with 264.84 and 186.71 q/ha, respectively. Similar trend has been observed for plant height also; and maximum height of 124.48 cm was attained by *C. ciliaris* followed by *C. setigerus* and *L. sindicus* with 104.52 cm and 89.95 cm, respectively (Table 1). *C. ciliaris* has provided 55.32 and 9.50% higher green forage yield over *L. sindicus* and *C. setigerus*, respectively. Therefore, high yielding genotypes of *C. ciliaris* and *C. setigerus* may be preferred for pasture development programme in district Jalore (Rajasthan). Rao and Singh (1994) reported that under Jodhpur conditions *C. ciliaris* produced higher dry matter yield, water and energy use efficiency than *C. setigerus*.

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Plant Germplasm Registration Notice*

The Plant Germplasm Registration Committee of ICAR in its XXXIInd meeting held on August 17th, 2015 at the ICAR-National Bureau of Plant Genetic Resources, New Delhi approved the registration of following 23 germplasm lines out of 43 proposals considered. The information on registered germplasm is published with the purpose to disseminate the information to respective breeders for utilization of these genetic stocks in their crop improvement programmes. Upon request, the developer(s)/author(s) is/are obliged to distribute the material for crop improvement programme of National Agricultural Research System.

1. ANR 38 (IC0613963; INGR15014), a Rice (*Oryza sativa* L.) Germplasm with Open Florets

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Rice (*Oryza sativa* L.) is a self-pollinating and self-fertilizing plant. This behavior is due to cleistogamous nature of rice spikelets which prevents out-crossing with the adjoining plants. This traditional trait is useful to maintain genetic purity, unique identity and homozygosity of rice genotypes. However, the cleistogamous nature is often a constraint in practical hybrid rice breeding wherein wider opening of spikelets or florets is desired to expose stigma to the maximum possible extent to allow landing of maximum number of pollen grains of the intended male parent. Similarly, protruding anthers are desirable in the male parent of the hybrid to shed copious amount of pollen on the targeted female parent or CMS line. External intervention like GA_3 spray application and rope polling etc are generally followed for achieving higher seed set during hybrid seed production. To facilitate higher pollen shedding by male plant and higher seed set on female (CMS) lines, identification and transfer of "Open florets" trait will be of considerable importance and utility. Though the "Open floret" trait leading to promiscuity in rice is quite scanty, we report its occurrence in a rice germplasm "ANR38" which is collected from the Bay islands (Gautam *et al.*, 2013).

Associated characters and cultivation practices: The ANR 38 rice germplasm is quite tall (185 cm) and photo-sensitive (186 days for maturity from seed to seed) with 6-8 tillers/plant, has long, erect and dark green leaves. It has strong culm with long panicles (28 cm), medium bold grains with grain length of 8 mm and width of 3.2 mm. It has higher test weight of 1000 grains (28 g) and brown colored lemma. Significant to mention, its florets remain sufficiently open for a longest time. The anthers are considerably protruded outside the spikelets and stigma is well exposed (Anonymous, 2014).

The genetic and molecular basis of open florets have been recently unravelled (Xiao *et al.*, 2014) the presently identified germplasm can be used as a donor for genetic transfer of this trait in both male and female parents of the intended hybrids for achieving higher seed set for the commercial viability and success of the rice hybrids.

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2. CNH-1102 (IC0611336; INGR15015), a Cotton (*Gossypium hirsutum* L.) Germplasm with High Ginning Outturn

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Cotton is commercially cultivated for its natural fibres called 'lint'. Lint is the most important industrial raw material. Cotton fibers are single-celled outgrowths from individual epidermal cells on the outer integument of the ovules. Certain epidermal cells produce outgrowths that continue to grow or lengthen up to 30 to 40 mm in length, lint hairs of fibre while other cells produce outgrowth similar to fibre but stop growing after attaining short length. These short epidermal hairs are known as fuzz. Cotton fibre development consists of four overlapping stages: fiber initiation, cell elongation, secondary wall deposition, and maturation. Cotton varieties and germplasm lines genetically differ in having relative proportion of long fibres (lint) or short hairs (fuzz). The long fibres after maturation (fibre with increase both in length and thickness, lint), can be easily removed from the seed surface by ginning. Ginning outturn is described as the percentage of lint obtained from a sample of seed cotton and is a useful indicator of the performance of a genotype. Genotypes with high ginning outturn are thus more preferable, because they yield more lint.

CNH-1102, an upland cotton genotype, is a selection developed from a random crossing of three *Gossypium hirsutum* (LRA5166, G. Cot. 10 and Bikaneri Nerma) and one *G. barbadense* (Suvin) genotypes. CNH-1102 is long staple cotton genotype with staple length 26 to 30 mm, fibre strength 20.7 to 24.4 g/tex and high average ginning outturn 40.9%. It is medium tall (90-110 cm), matures in 160-165 days and synchronous in boll bursting. During the evaluation under All India Coordinated Cotton Improvement Project (AICCIP) in 2007-08 and 2008-09, it has shown superiority for ginning outturn percent and fibre quality traits specially fibre strength over rest of the genotypes including best zonal check, Sahana and local check varieties. CNH-1102 has recorded seed cotton yield of 1359-1573 kg/ha.

In all 12 AICCIP trials during 2007-08 and 2008-09, CNH-1102, was identified as the best/ top ranking genotype for ginning outturn and fibre strength. In addition to fibre strength and length, it was also found to be resistant to bacterial leaf blight, tolerant to jassids and has wide adaptability.

CNH1102 recorded highest average GOT of 40.9% that ranged from 37.4 to 44.3%, an increase of above 14.88% over the best zonal check variety Sahana and 9.36 over the local check varieties at 4 locations in the advance South Zone trials of AICCIP in 2008-09. The average fibre strength was 24.0 g/tex with an increase of above 21.2% over the best zonal check variety Sahana and 7.14 over the local check varieties. While the average staple length was 29.0 mm which is higher by 5.5% over the zonal check Sahana and 3.2% over the local check varieties. The strength/length ratio of CNH-1102 was 0.83 indicating its fibre suitability and superiority for high speed spinning in comparison to zonal check Sahana (0.72) and local check (0.79) varieties.

Ginning outturn is one of the most important economic trait and major component of cotton economic lint yield. Cotton ginning outturn is much used measurement in cotton production, marketing and ginneries. Crop growing conditions and location plays major role affecting ginning outturn. However, it has been observed that the ginning outturn is a genetically controlled character. With the advances in molecular markers studies and development of saturated genetic linkage maps, it is understood that several QTLs influence the ginning outturn in cotton. The number of seeds/ boll is a component of cotton yield, lint yield (ginning outturn) and fiber quality. Both cultivar and environment contribute to the variation in the number of seeds/boll and also ginning outturn (Hossam, 2010). Ginning outturn is a useful indicator for the performance of a genotype (Waghmare *et al.,* 2009, Joubert *et al.*, 2002). Since cotton lint is considered to be most economic and valued from textile industries and fabrics manufacturing, measurement of lint as an index or proportion helps to identify varieties/genotypes producing more lint.

Fibre quality, specifically fibre strength is the most valued parameter alongwith length considered in yarn spinning and fabric making. Stronger the fibre, least will be the neps and better finish quality of fabrics (Hossam, 2010). Availability of germplasm with combination of both these important traits is rare. Thus, the genotypes

with combination of ginning outturn or lint yield and fibre quality would serve as better genetic stocks for further improvement of both the characters. In addition, the genotype is resistant to bacterial leaf blight disease most severely found in all cotton growing areas, tolerant to jassids and has wide adaptability.

Table 1. Features of CNH 1102

Characters	CNH 1102
Boll weight (g)	3.63 (2.4-5.1)
No. of bolls/plant	25.7(14.1-38)
Seed index (g)	7.85
Ginning Outturn (%)	40.9 (34.9-44.3)
Lint index (g)	5.43
Seed Oil content (%)	17.3
No. of monopodia	1.0
No. of sympodia	20.8
Seed cotton yield (q/ha)	13- 20
Days to maturity (days)	160-165
Fibre quality	
Fibre length (at 2.5% SL)	28.3mm (26.2-29.7)
Uniformity ratio	52
Micronaire	3.9 (3.7-4.0)
Maturity (%)	74
Bundle strength (g/tex)	22.4 (20.7-24.4)
Elongation (%)	6.3

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3. CNA-1051 (IC0613964; INGR15016), a Cotton (*Gossypium arboreum* L.) Germplasm with Distinct Yellow Top Leaves

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Cotton is an important commercial crop mainly grown for natural fibres and seed oil. It is cultivated in more than 80 countries of the world which include Australia, America, Africa, China, India, Pakistan and Uzbekistan. Among the four cultivated species, diploid species, namely *Gossypium arboreum* and *G. herbaceum*, are mainly cultivated in India, China and Pakistan. South East Asia is considered as the home of *G. arboreum*, possessing wide adaptability and grown on range of soil types and low input conditions. *Desi* cotton varieties are relatively tolerant to biotic (insect pests and diseases) and abiotic stresses (drought and salt stresses), synchronous in maturity and possess consistent fibre properties.

Mutation breeding method is known for creating additional genetic variability and exercising selection for desirable type. Although most of the mutations are usually undesirable, mutations with desirable traits and also the types that did not exist in the gene pool do occur in the population.

G. arboreum variety Y1 was very popular in the Khandesh tract of Central Zone despite its poor yield. Variety Y1 was used for mutation studies. Dry delinted seeds of var. Y1 were subjected to mutagenic treatments of gamma rays (150, 200, 250 and 300 Gy) and 6 hours presoaked seeds in distilled water to ethyl methane sulphonate (0.1, 0.2 and 0.4% for 2 and 4 hours

treatment). The treated seeds were planted in the field, selfed, harvested separately and grown as single plant progenies in M_2 (Waghmare and Koranne, 2000).

A stable virescent chlorophyll mutant with yellow top leaves (3-5 leaves) and remaining normal green leaves were identified in EMS 0.1% (6h) treatment in M_2. The mutant plant identified in M_2 was raised as single plant progeny in M_3 and it showed complete uniformity for phenotypic traits in M_3 and subsequent generations. The mutant was rigorously evaluated in M_5 generation. Cross between the virescent mutant and control var. Y1 was attempted to study the inheritance of the changed character.

Virescent chlorophyll mutant had average plant height of 114 cm slightly less than that of parent variety Y1. The mutant plant type was similar to parental variety Y1 except yellow leaves (3-5) at the growing top which is a distinguishing and unique trait of this mutant. Boll shape and size, leaves shape and colour and fibre quality traits of mutant were comparable to parental variety Y1. The mutant is unique for its yellow young leaves at the growing top that become normal green with age as plant grows and distinguishable in the population since early seedling stage until cessation of plant growth. After cessation of plant growth, matured top leaves can barely be distinguished from other green leaves on the plant.

Except the morphological features, CNA-1051 has similar economic features as that of parental variety Y1. It matures in 160-165 and is synchronous in boll bursting. Seed cotton yield of this mutant varies from 18 to 65 g/plant and12-14 q/ha depending on climate and soil types. The ginning outturn which reflects proportion of lint to seed cotton yield is 35.6%. Its fibre characteristic features are as given (Table 1).

The inheritance of changed character has been studied by attempting crosses with parental var. Y1. The F_2 and $BCa+_1$ segregation ratio revealed monogenic recessive gene inheritance of this virescent mutant.

The virescent chlorophyll mutation in CNA-1051 is very unique mutant germplasm; it is stable and has high heritability. The mutant is so distinct that can be identified in early seedling stage until plant maturity. The unique features of this line can be utilized as marker trait. The mutant would also serve the purpose for basic studies on development pathway of chlorophyll in diploid cotton.

Table 1. Features of CNA 1051 – a virescent mutant

Plant height (cm)	114.0
No. of monopodia	1.4
No. of sympodia	17.20
Internode length (cm)	5.23
Average seed cotton yield /plant (g)	12- 14
Average Boll weight (g)	2.56
Ginning Outturn (%)	35.6
Seed Index (g)	5.82
Lint Index (g)	3.22
Staple length (mm)	24.1
Uniformity ratio (%)	50
Micronaire (µg/inch)	5.2
Fibre Bundle Strength (g/tex)	21.3
Fibre Maturity (%)	72
Fibre Elongation (%)	4.6

In our view, it is a robust genetic stock with comparable and acceptable fibre quality and economic traits, ginning outturn (35.6%) with yellow growing top leaves feature which makes it a unique mutant or germplasm genetic stock.

Virescent leaves are an important character of plant that may be useful in genetic, physiological studies and breeding in cotton (Song *et al.*, 2012). Virescent mutations occur in a wide range of flowering plants, including cotton (Kohel 1967, 1974 and 1983). Benedict and Kohel (1968) have shown that a virescent cotton mutant shows a high rate of CO_2 assimilation. Further, it is reported that yellow virescent cotton leaves had a high photosynthetic rate, low amount of PEP carboxylase activity (which is typical of most plants possessing a Calvin Cycle, and an absence of lamellar aggregation into grana in the chloroplasts Benedict and Kohel (1970). The virescent mutants in cotton have a decreased amount of chlorophyll and carotenoids but a normal level of ribulose diphosphate carboxylase (Kohel and Benedict 1971). To date, more than 30 virescent and 2 albino mutants have been genetically characterized in the tetraploid cotton species and about 26 virescent nuclear genes have been identified in cotton (Song *et al.*, 1968). CNA 1051 is the first of its kind of virescent mutant being reported and registered as genetic stock in diploid cotton *G. arboreum*. It requires to be further characterized for structural, physiological and functional aspects.

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4. NRC-109 (IC0612435; INGR15017), a Soybean (*Glycine max* L. Merril) for Lipoxygenase-2 Free Soybean with Early Maturity (85 Days)

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The off-flavour associated with soy products is one of the major deterrents in the widespread acceptance of soyfood. This is due to the hexanal compounds released by the catalytic oxidation of polyunsaturated fatty acids in the oil fraction by seed lipoxygenase enzyme. When the seeds are subjected to grinding for making any soy product, lipoxygenase comes in contact with its substrate (polyunsaturated fatty acids) and thereby triggers the catalytic oxidation in the presence of oxygen. In fact, lipoxygenase in soybean seed exists in three isozymic forms – lipoxygenase-1, lipoxygenase-2 and lipoxygenase-3. Presence of each of the isozymes is controlled by single dominant genes, *i.e.* Lx1, Lx2 and Lx3 and their absence is ascribed to the corresponding null alleles (lox1, lox2 and lox3). Soy preparations made from seeds of lipoxygenase-free genotypes are better accepted due to production of low levels of hexanal compounds (Davies *et al.*,1987) . Though lipoxygenases are heat labile, heat inactivation employed at industrial level not only incurs extra cost, but also affects the solubility and functionality of proteins. In India, none of the soybean varieties released so far is free from any of the three lipoxygenases. Lipoxygenase-2 is mainly responsible for generation of off-flavour imparting n-hexanal in soybean products. Genetic removal of lipoxygenase-2 from the seeds has been shown to significantly improve the flavour of soy products. Therefore, bringing lipoxygenase-2-free soybean under cultivation and making the seeds available for soy processors can boost utilization of soybean in food. In view of this pressing need of the soy processing industry, a breeding programme aimed at the development of lipoxygenase-2-free soybean genotypes was initiated at the Directorate of Soybean Research, Indore.

The authors successfully developed lipoxygenase-2 free genotype INRC109 by crossing Samrat and PI086023. Samrat is a farmers' variety of soybean cultivated widely in Central India. PI086023 is a source of lox2 allele which was procured from the United States Department of Agriculture. Its plant type is agronomically poor and not adaptable to Indian conditions. The crossing programme was carried out up to F7 generation. The selection for null lipoxygenase-2 plants in each generation was made by rapid assay in the seeds, according to Suda *et al.,* (1995). Validation of null lipoxygenase-2 plants in the advanced generations of Samrat × PI086023 was performed using null allele-specific marker recently designed by Shin *et al.,* (2012) from the sequence analysis of lox2 gene (null allele of lipoxygenase-2) of Korean cultivar Jinpumkong 2. DNA amplification using this gene-specific marker was confirmed by deploying it on null allele of lipoxygenase-2 from genotype PI086023 in the present study. For this purpose, genomic DNA extracted from the young leaves of NRC109, NRC110, Samrat (the recipient) and PI086023 (the donor of null allele of lipoxygenase-2) using the CTAB method and used as template for amplification using the primer specific for the null allele of lipoxygenase-2. Amplicons of 769 bp size were observed in NRC109 , similar to the donor parent PI086023 and no amplicon was seen in Samrat, the lipoxygenase-2-positive parent. This confirmed the

transfer of null allele of lipoxygenase-2 in NRC109. NRC109 yielded bold seeds with yellow seed coat and brown hilum with 100 seed weight of 16.5g. NRC109 is early maturing (85 days), a character desired by the farmers in Central India, with yield potential of 2.6 tonnes/ha. The germplasm line developed can be very useful in the enhancement of soybean in food uses. This line will be very useful as a source of null lox2 allele in improved agronomic background as pre-breeding line. Besides, this genotype possess the desirable trait of earliness.

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5. CSIR-IHBT-Gr-E-3(HIM Peace) (IC0613966; INGR15018), a Gerbera (*Gerbera jamesoni* Bolus ex Hook. f) for Double Flower Shape, Medium Size (~8 cm Flower Diameter), White Flower Colour (RHS 155D)

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Gerbera (*Gerbera jamesonii*) is an important commercial cut flower of the trade and there is an increasing demand of new types of varieties for export as well as in domestic markets. In this context, with an aim of varietal development, selective breeding of gerbera was done and hybrid F_1 genotype (CSIR-IHBT-Gr-E-3) of gerbera was developed through controlled crossing program and subjected to *in vitro* micro-propagation to achieve quick multiplication. Rooted plantlets were hardened and transferred for cultivation in soil.

Morpho-agronomic characteristics: Characterization of hybrid F_1 genotype under field conditions with respect to floral traits and evaluation for agronomic performance provides evidence about promising gerbera genotype CSIR-IHBT-Gr-E-3 which has double flower shape of medium size (flower diameter of 7.9 cm) and is white in color (RHS 155D). The genotype has high micro-propagation potential for commercial utilization (Singh *et al*., 2015).

Associated characters and cultivation practices: In the field experiment conducted during 2013-14 and 2014-15, based on mean performance of hybrid gerbera genotypes with the respective parents, CSIR-IHBT-Gr-E-3 was superior to at least one parental genotype for traits plant spread in a year (cm), leaf length (cm) and leaf width (cm), while it was *at par* to parental genotypes for the other traits (Table 1).

Table 1. Mean performance of gerbera genotypes over two years

S. No.	Source of variation	IHBT-Gr-01		IHBT-Gr-07		CSIR-IHBT-Gr-E-3	
		2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
1.	Leaf number per plant/year	31.25	33.15	32.05	33.05	29.3	31.85
2.	Plant spread (cm)/year	57.75	58.45	48.95	49.45	63.25*	63.8*
3.	Number of suckers per plant/year	2.45	4.45	2.7	6.25	2.75	5.7
4.	Leaf length (cm)	32.55	32.60	23.0	23.75	34.25*	33.425*
5.	Leaf width (cm)	11.55	11.3	7.17	7.075	12.87*	11.975*
6.	Flower number per plant/year	21.15	22.95	17.6	19.55	18.8	21.8
7.	Scape length (cm)	34.6	33.55	50.05	49.05	39.3	40.75
8.	Flower diameter (cm)	8.22	8.25	7.55	7.775	8.05	8.225

*Significant at p = 0.05

Reference

Singh S, Raja Ram, S Kaundal, A Sharma, A Kumar and D Dhyani (2015) Field performance and differential response of micro-propagated potential F_1 genotypes of *Gerbera jamesonii*. *American Journal of Experimental Agriculture* DOI: 10.9734/AJEA/2016/20653.

6. CSIR-IHBT-Gr-23 (HIM Glow) (IC0613967; INGR15019), a Gerbera (*Gerbera jamesoni* Bolus ex Hook.f.) for Double Flower Shape. Standard Size (>10 Cm Flower Diameter). Yellow Orange Flower Color (RHS 16C)

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Gerbera (*Gerbera jamesonii*) is an ideal cut flower and ranks fifth among top cut flowers in the international market. New cultivars of gerbera are developed by hybridization of diverse types to create novel genotypes. In this regard, selective breeding of gerbera was done and hybrid F_1 genotype CSIR-IHBT-Gr-23 of gerbera was developed through controlled crossing program and subjected to *in vitro* micro-propagation to achieve quick multiplication. Rooted plantlets were hardened and transferred to sleeves for cultivation in soil.

Morpho-agronomic characteristics: Characterization of hybrid F_1 genotype CSIR-IHBT-Gr-23 under field conditions with respect to floral traits and evaluation for agronomic performance provides evidence about this promising gerbera genotype which has double flower shape of standard size (flower diameter of 10.8 cm) and is yellow orange in color (RHS 16C). The genotype also has high micro-propagation potential for commercial utilization (Singh *et al*., 2015).

Associated characters and cultivation practices: In the field experiment conducted during 2013-14 and 2014-15 significant genotypic variations were recorded for the agro-morphological and floral traits based on mean performance of hybrid gerbera genotype with the respective parents; CSIR-IHBT-Gr-23 was superior to at least one parental genotype for traits leaf width (cm), flower number per plant/year and scape length (cm) while it was *at par* to parental genotypes for the other traits.

Reference

Singh S, Raja Ram, S Kaundal, A Sharma, A Kumar and D Dhyani (2015) Field performance and differential response of micro-propagated potential F_1 genotypes of *Gerbera jamesonii*. *American Journal of Experimental Agriculture* DOI: 10.9734/AJEA/2016/20653.

Table 1. Mean performance of gerbera genotypes over two years

Source of variation	IHBT-Gr-02		IHBT-Gr-03		23-1	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
Leaf number per plant/year	20.85	23.7	22.75	24.45	25.4	28.65
Plant spread (cm)/year	66.05	66.55	66.25	66.8	65.85	66.55
Number of suckers per plant/year	2.3	4.5	2.45	5.15	2.8	5.75
Leaf length (cm)	37.2	37.3125	36.35	35.6875	38	37.46667
Leaf width (cm)	12.175	11.675	14.675	14.6	16.2*	15.22*
Flower number per plant/year	13.95	16.35	12.75	14.75	23.5*	23.4*
Scape length (cm)	32.8	31.95	32.6	31.5	45.4*	44.75*
Flower diameter (cm)	9.65	9.725	9.35	9.55	10.85	10.75

*Significant at p = 0.05

7. WH 1127 (IC0610417; INGR15020), a Wheat (*Triticum aestivum* L.) Germplasm with High Gluten Index (86%)

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The genotype WH 1127 is a selection from a cross RL 6043/4/NAC// PASTOR/3/BABAX. The proposed genotype WH 1127 was evaluated in the Advanced Varietal Trial under timely sown rainfed conditions in the North Western Plain Zone of the country during the year 2012-13. This genotype possessed better quality characters in terms of high gluten index, i.e., 86%.

Morpho-agronomic characteristics: This genotype has semi-erect growth habit dark green foliage and average plant height of 101 cm. The mean days to heading are about 111 days and maturity is about 153 days under rainfed conditions. Its grain is amber in colour with about 41 g 1000-grain weight. Ear shape is tapering with medium ear density and hairy glumes.

Associated characters cultivation practices: This genotype is suitable for cultivation under timely sown rainfed areas of the NWPZ of the country and resistant to lodging.

8. DHTW-60 (IC036761A; INGR15021), a Wheat (*Triticum aestivum* L.) Germplasm with Heat Tolerance

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Drought and heat are two abiotic stresses which affect wheat yields drastically. In India, nearly 80% wheat is cultivated under irrigated conditions, 66% of it receives only partial (1-2) irrigations, and the remaining 20% is grown under rainfed environments. Heat stress on the other hand is affecting around 13.5 million ha grown under wheat in India (Garg *et al*., 2013; Sharma *et al.*, 2014). Both drought and heat stress are responsible for 20 – 30% reduction in grain yield.

Genotype DHTW 60 (IC 36761A) is a unique genotype having tolerance to heat tolerance. It was evaluated for heat stress tolerance under field conditions by timely (non-stress) and late (heat stress) sowing as well as under temperature controlled glass house condition during 2011-12. The mean minimum and maximum temperature during grain growth period was higher by 2.4ºC and 2.7ºC under late sown field condition compared to timely sown. Under glass house conditions, temperature in one chamber was maintained at ambient whereas in other chamber it was higher by 2.0ºC. The genotype registered 2.2% reduction in 1000 grain weight under high temperature stress condition and heat susceptibility index was 0.20 (Table 1).

Multi location evaluation for heat tolerance at 3 locations: DHTW-60 was evaluated for heat tolerance at three locations; Hisar, Kanpur and Pune during 2012-13. The mean minimum temperature during grain growth period under late sown condition was higher by 1.6 to 3.6ºC and mean maximum by 2.0 to 4.3ºC. The data pooled over location received 14% reduction in thousand grain weight and 6.6% in grain yield. The Pooled heat susceptibility index was 0.39. Location wise genotype was tolerant to heat stress at Hisar and Kanpur (Table 2).

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Table 1. Data on 1000 grain weight (TGW) and reduction in grain filling duration (GFD), grain weight/spike (GW) and TGW over control under high temperature stress conditions and heat susceptibility index

Genotype	TGW	Reduction (%)			Heat Susceptibility index
		GFD	GW	TGW	
DHTW60 (IC 36761A)	37.0	16.9	9.7	2.2	0.20
HTW 11 (Check)*	38.7	6.0	-0.03	4.39	0.63

*Registered Genetic Stock

Table 2. Heat susceptibility index at different locations

Genotype	Hisar	Kanpur	Pune	Mean
DHTW 60 (IC 36761A)	0.39	0.11	1.10	0.39
HTW 6(Check)*	0.66	1.07	1.23	1.09
HTW 11(Check)*	0.80	0.65	0.85	1.02

*Registered Genetic stock

9. IC536140 (IC0536140; INGR15022), a Wheat (*Triticum aestivum* L.) for Rust Resistance with 3 Minor/APR Genes *viz.*, *Lr*34/*Sr*57/*Yr*18/*Pm*38; *Lr*46/*Sr?*/*Yr*29/*Pm*39 and *Lr*67/*Sr*55/*Yr*46/*Pm*46

and

10. EC573562 (EC573562; INGR15023), a Wheat (*Triticum aestivum* L.) for Rust Resistance with 3 Minor/APR Genes *viz.*, *Lr*34/*Sr*57/*Yr*18/*Pm*38; *Lr*46/*Sr?*/*Yr*29/*Pm*39 and *Lr*67/*Sr*55/*Yr*46/*Pm*46

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Wheat is one of the most important cereal crops for food security and rust diseases (Stem/black rust (caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks, and Henn.) brown or leaf rust (caused by *Puccinia triticina* Eriks.) and stripe/yellow rust (caused by *Puccinia striiformis* Westend.) continually pose a threat to wheat production at national and international level. Resistant cultivars are the cheapest, most reliable and environmentally safest way to control the rust diseases. Historically, many genes were deployed to provide resistance to rust diseases. However, most of the rust resistance genes, although initially effective were often short lived due to the ability of the pathogen to become virulent after single step mutations. In wheat breeding programmes, stem, leaf and stripe rust resistance could be achieved through several approaches. Major gene resistance (race specific) is achieved through conventional or backcross approach, or alternatively, breeders could select against race specificity (race non-

specific) and accumulate genes for slow rusting or adult-plant resistance in a cultivar.

Minor genes are the slow-rusting adult plant resistance (APR) type that confers partial resistance in race non-specific manner to one or multiple rust diseases. Slow rusting, race non-specific or partial resistance was defined by Parlevliet (1975). Slow rusting is a type of resistance where disease progresses at a retarded rate, resulting in intermediate to low disease levels against all pathotypes of a pathogen. The concept of race non-specific, horizontal resistance was widely used by Caldwell (1968) in breeding leaf rust resistant wheat varieties. APR is commonly detected at the post-seedling stage and often as field resistance, but seedling susceptible, although some APR genes are sensitive to varying temperature and light conditions. So far, five rust resistance genes (*Lr*34/*Sr*57/*Yr*18/Pm38, *Lr*46/*Sr*?/*Yr*29/*Pm*39, *Lr*67/*Sr*55/*Yr*46/*Pm*46, *Lr*68/*Sr*?/*Yr*?/Pm? and *Sr*2/*Lr*27/*Yr*30/*Pm*?) were identified and catalogued as minor genes. These genes are pathotype nonspecific adult plant resistance with pleiotropic association with stem and stripe rust resistance along with powdery mildew.

Table 1. Rust reactions of wheat accessions at different environments in comparison to resistant and susceptible check

Sl. No	Accession No./Checks	Summer - 2011 Wellington		Rabi 2011-12 & Summer- 2012 Wellington Leaf Rust - Adult plant reaction			Rabi 2011-12 & Summer - 2012 Wellington Leaf Rust - Seedling reaction test					Rabi 2013-14 Shimla		
		Leaf Rust	Stem Rust	I	II	III	17	77A	77-5	77-7	77-8	Stripe rust	Stem rust	Leaf rust
1	IC536140	10MR-MS[#]	F	MR-MS	10 MR-MS	20S	3	X	X	3	3	F	F	F
2	EC573562	10MR-MS	F	MR	5MR	20MR	3	3,3$^+$	3	X	3,3$^+$	F	F	F
3	Resistant Check - Diamond Bird (Genetic stock for Lr34)	30MS-S	20MR-MS	10MR-MS	20MR-MS	40MS-S	3$^+$	3,3$^+$	3$^+$	3$^+$	3,3$^+$	20S	F	F
4	Resistant Check - Pavon 76 (Genetic stock for Lr46)	40MS-S	40MS-S	10MS-S	20MS-S	40S	3,3$^+$	3$^+$	X	3,3$^+$	3$^+$	20S	F	F
5	Resistant Check - RL6077 (Genetic stock for Lr67)	30MS-S	40MR-MS	20MR-MS	30MR-MS	40S	3$^+$	X	3,3$^+$	3$^+$	3,3$^+$	40S	F	F
6	Susceptible check (Agra Local for leaf & stem rust and) A-9-30-1 for stripe rust at Shimla)	80S	60S	40S	80S	100S	3$^+$	3,3$^+$	3$^+$	3,3$^+$	3$^+$	80S	F	F

[#] F=Free; R=Resistant; MR=Moderately resistant; MS= Moderately Susceptible; S=Susceptible; T=Traces

Nearly 20,000 wheat accessions conserved by the National Bureau of Plant Genetic Resources (NBPGR), rejuvenated at Indian Agricultural Research Institute (IARI), Regional Station, Wellington through National Off-season nursery facility were initially screened for the presence of leaf tip necrosis (LTN), a phenotypical trait (morphological marker) linked to the APR genes *Lr*34+, *Lr*46+ and *Lr*67+. Initially 2,200 accessions were selected in summer, 2011 which were again screened in *rabi*, 2011-12 and summer, 2012 at Wellington, of which, 36 wheat accessions which showed prominent necrotic tipping on the leaves and resistant rust reaction in field (Table 1) were subjected to Seedling reaction test (Table 1) and molecular marker analysis. Since this trait (LTN) is environmentally sensitive, use of molecular markers is the reliable option to confirm the presence of these

minor genes. These accessions were again tested for rust diseases at Shimla (Table 1) for stripe rust. Out of the total 36 wheat accessions with LTN when tested with molecular markers (*Lr*34 (Lagudah *et al*., 2006); *Lr*46 (Suenaga *et al.*, 2003); *Lr*67 (Hiebert *et al.*, 2010)), two lines IC536140 and EC573562 carried all the three APR genes.

At field level, these three accessions showed resistant reaction to stem and stripe rusts, while displayed variable reaction to leaf rust, but within the permissible limit. As expected seedling reaction test showed susceptible reaction, a characteristic feature of minor/APR genes. Pyramiding of these minor genes provide durable and non-specific adult plant resistance. These slow-rusting gene(s) when utilized in combinations in a particular varietal background confer high levels of resistance to leaf, stem and stripe rust and are also expected to be durable. Hence, pyramiding of these minor genes *Lr34+*, *Lr46+* and *Lr67+* are expected to confer resistance that represent an attractive option to breeders for durable multi-pathogen resistance to leaf rust, stripe rust and stem rust.

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11. CNA-407 (NLL-Spotted Petals) (IC0613960; INGR15024), a Cotton (*Gossypium arboreum* L.) Germplasm with Narrow Leaf Lobes, Spotted Petals and Brown Lint

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A brown linted genetic stock, CNA-407 (INGR15024) of *Gossypium arboreum* was developed by pedigree selection. The fiber traits of this genotype are presented in the table.

Economic features and fiber quality traits	
Average Seed Cotton Yield/Plant (g)	105.00
Average boll weight (g)	2.30
Ginning Outturn percentage	33.50
Seed Index (g)	7.50
Lint Index (g)	3.90
Staple length (mm)	27.30
Uniformity ratio (%)	51.00
Micronaire (µg/inch)	3.80
Fiber Bundle strength (g/tex)	19.80
Fiber maturity (%)	58.00

The stock has following morphological characters taken based on DUS guidelines which include pigmented okra leaf, yellow flower petal with petal spot, yellow pollen colour, pigmented anther filament, red and ovate boll, pitted boll surface, pointed boll tip and light brown seed fuzz colour. Colour fastness of lint was tested as per AATCC test resulting in rating of 5 showing fair stability of colour on washing.

12. CNA-407 (NLL-Spottless Petals) (IC0613961; INGR15025), a Cotton (*Gossypium arboreum* L.) Germplasm with or Narrow Leaf Lobes, Spotless Petals and Brown Lint

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A brown linted genetic stock, CNA-407 (INGR15025) of Gossypium arboreum was developed by pedigree selection. The fiber traits of this genotype include 26.8mm staple length, 51% uniformity ratio, 4.6 micronaire, 19.2 g/tex fibre tenacity and 67% fibre maturity coupled with brown colour lint. The stock is characterized by morphological characters such as okra leaf shape, yellow flower petal without spot, yellow pollen colour, absence of anther filament colouration, red and ovate boll, pitted boll surface, pointed boll tip and light brown seed fuzz colour. Colour fastness of lint was tested as per AATCC test resulting in rating of 5 showing fair stability of colour on washing.

13. IL-13-106 (IC0613360; INGR15026), a Berseem (*Trifolium alexandrinum* L.) Germplasm with Black Seeds and Pentafoliate Leaf

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Berseem (Egyptian clover, *Trifolium alexandrinum* L.) is one of the most important winter season fodder crop. Because of narrow genetic base of the crop, interspecific hybridization using embryo rescue technique was attempted to generate variability and transfer of traits like disease resistance, quality, longevity, yield etc. Several interspecific hybrids of *T. alexandrinum* with different related wild species were developed at Indian Grassland and Fodder Research Institute (Roy *et al.*, 2004; Malaviya *et al.*, 2004; Kaushal *et al.*, 2005). Hybrids of *T. alexandrinum* × *T. apertum* (both parents were yellow seeded and trifoliate) were generated which showed segregation for black seeded and pentafoliate traits in F_2 generation. Selected plants were advanced and by recurrent selection line combining pentafoliate with black seeds (RHS no. black 202A) were developed. Thus, a novel genetic stock, *viz.*, black seeded pentafoliate berseem, was identified. Progeny lines of this novel genetic stock were planted to analyse the stability of these two traits. Progeny lines showed 100% penetrance for both traits while >95% expressivity was observed for pentafoliate trait. Due to black colour the seeds are expected to contain higher tannin content in its seed coat, which may confer better tolerance to pathogens in the early stages of seedling development. Additionally, the pentafoliate nature is a distinct morphological trait and can be used in breeding programme to enhance productivity and studies in leaves morphogenesis. Further to mention that black (dark tan) seeded pentafoliate berseem has never been reported. In future, it might serve as a marker in various intra and interspecific crosses. This novel genetic stock of berseem with black-seed and pentafoliate leaves could potentially be used for basic studies as well as genetic improvement programme in berseem crop (Anonymous 2013).

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14. SPV-2018 (IC0612063; INGR15027), a Sorghum (*Sorghum bicolor* (L.) Monech.) Germplasm with High Digestibility (IVOMD %), Low Lignin Content and Brown Midrib (*bmr*)

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The significance of brown midrib mutation in sorghum is that it greatly reduces lignification and cell-wall concentration, increases digestibility, and increases voluntary intake of feed by ruminants. Genetic control of the lignification process through use of brown midrib (*bmr*) mutations has offered the most direct and productive approach to reducing lignin concentration and increasing digestibility of sorghum. Digestibility of fodder is the most important forage quality parameter which helps in better absorption of the feed taken in by the animal. It is estimated that a 1% increase in digestibility increases milk yield by 5%, resulting in higher income for farmers (Rao and Hall, 2003; Kumaracharyulu *et al.*, 2014).

SPV 2018 was developed through pedigree method of breeding by crossing between SPV 462 and the *bmr* germplasm line, IS 21891 during rabi 2003-04 at Directorate of Sorghum Research, Hyderabad. SPV 462 is a most popular variety which was a released as a variety in the states of Tamil Nadu and Andhra Pradesh (PSV 1) while IS 21891 is a brown midrib source carrying the gene *bmr 8*. Agronomically superior derivatives from this cross were selected and advancement of segregating generations was done from F_3 till F_7 between the period 2004-2007.SPV 2018 is a tan plant with green leaves and brown midrib. Panicle is semi-compact with elliptic shape and medium bold seeds. After stabilization, it was assessed for its yield and quality in station trials during 2007-08 before testing under All India Coordinated Sorghum Improvement Project Trials during 2009 and 2010. Station trial results for IVOMD indicated the superiority of SPV 2018 i.e., (SPV462 × IS 21891)-3-1-1-1 (52.2%) over the test entries. It was also promising for Relative Fodder Value and was the best among the test entries for this character (Umakanth *et al*., 2014).

During 2009, SPV 2018 with an IVOMD of 52% had recorded a superiority of 6% for IVOMD (%) over the national check CSV 23 (49%) and local check (49%). During 2010, with an IVOMD of 42.3%, it had recorded a significant superiority of 9% over the national check CSV 23 which has shown 38.7% and 5% over the local check (40.3%). It also had 14% significant superiority for crude protein content and lower lignin content over the national check variety CSV 23.

SPV 2018 offers promise for increased milk yield owing to superior stover quality in terms of low lignin content and higher *in vitro* organic matter digestibility. Apart from its use for increasing milk yields, this line offers promise as a lignocellulosic biofuel feedstock because of higher yield of fermentable sugars during pretreatment and enzymatic saccharification owing to its low lignin content. Earlier studies have indicated that brown midrib mutants significantly increase conversion rate in the lignocellulosic bioenergy process. This variety is suited to be grown under *Kharif* rainfed situation across India.

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15. VRP-147 (IC0598281; INGR15028), a Garden pea (*Pisum sativum* L.) Germplasm with Resistance to Downy Mildew and Rust

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Garden pea is main winter season crop and occupying about 4.6% of total vegetable area and about 2.3 % of total vegetable production. Uttar Pradesh alone contributes about 45.4% of total pea production in India. Diseases such as powdery mildew, downy mildew and rust are the major limiting factors in pea production especially for mid (flowering start 45 DAS) and late duration (flowering start 55 DAS) varieties. The losses in yield in a 100% infected crop by powdery mildew were estimated to be 21-31% in pod number and 26-47% in pod weight. Although these diseases can be controlled very effectively through fungicidal treatment but disease control by chemical means fetch more cost to its production and also it is hazardous to human health and the ecosystem. So, cultivation of resistant varieties is one of the practicable options for the management of the diseases because genetic resistance is safe, reliable, effective and cost-free alternative to ensure a healthy crop. So, keep in mind the above problems, experiment was laid out for screening of available germplasm lines at IIVR against powdery mildew, downy mildew and rust.

During 2008-09, a set of 410 germplasm lines were screened for resistance against powdery mildew, rust and downy mildew. Out of them 85 lines were resistance to powdery mildew and 9 were resistance to rust while five lines (VRP-233, VRP-393, VRP-343, VRP-147 and NO-17) were resistance to both the diseases. However, the incidence of downy mildew was not noticed during the period. Same set of germplasm lines (410) were again sown in 2009-10 under field conditions and observations were made on selected germplasm lines which comprises of resistance and susceptible for powdery mildew/downy mildew/rust. A high disease pressure was observed in powdery mildew and rust but a moderate level of downy mildew severity was noticed (20-26%). Among the lines screened, VRP-343, VRP-393, NO-17, VRP-147, NO-110 and VRP-233 showed resistance to powdery mildew and rust while VRP-343 and VRP-147 recorded resistance to all three diseases.

On the basis of two year field study, a set of different lines resistance to powdery mildew, rust and downy mildew along with suitable susceptible checks were tested under artificial inoculation conditions during 2012-13 and 2013-14. For artificial inoculation, the spores of all the diseases were collected from pea field in the last week of February and sprayed (approx. 10^6 spores/ml) on all the germplasm lines uniformly in polyhouse. The genotypes VRP-343 and VRP-147 were found resistant for all the three diseases whiles susceptible check showed 100 per cent disease incidence and the results were consistent while comparing previous experiments.

Morpho-agronomic characteristics: VRP-147 is a late maturing genotype. It takes 65-66 days for 50% flowering. Flowering starts after 17.4 nodes. Pod length is 5.5 cm and width is 1.42 cm. Pods are green in colour and slightly curved. Number of pods/plant is 24.5 and 10 pod weight is 44 g and each pod has 6.2 seed. Yield/plant is 98 g and shelling percent is around 47.2. The total sugar is 3.80 %.

16. VRP-343 (IC0598280; INGR15029), a Garden Pea (*Pisum sativum* L.) Germplasm with Resistance to Powdery Mildew

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Garden pea is main winter season crop and occupying about 4.6% of total vegetable area and about 2.3 % of total vegetable production. Uttar Pradesh alone contributes about 45.4% of total pea production in India. Diseases such as powdery mildew, downy mildew and rust are the major limiting factors in pea production especially for mid (flowering start 45 DAS) and late duration (flowering start 55 DAS) varieties. The losses in yield in a 100% infected crop by powdery mildew were estimated to be 21-31% in pod number and 26-47% in pod weight. Although these diseases can be controlled very effectively through fungicidal treatment but disease control by chemical means fetch more cost to its production and also it is hazardous to human health and the ecosystem. So, cultivation of resistant varieties is one of the practicable options for the management of the diseases because genetic resistance is safe, reliable, effective and cost-free alternative to ensure a healthy crop. So, keep in mind the above problems, experiment was laid out for screening of available germplasm lines at IIVR against powdery mildew, downy mildew and rust.

During 2008-09, a set of 410 germplasm lines were screened for resistance against powdery mildew, rust and downy mildew. Out of them 85 lines were resistance to powdery mildew and 9 were resistance to rust while five lines (VRP-233, VRP-393, VRP-343, VRP-147 and NO-17) were resistance to both the diseases. However, the incidence of downy mildew was not noticed during the period. Same set of germplasm lines (410) were again sown in 2009-10 under field conditions and observations were made on selected germplasm lines which comprises of resistance and susceptible for powdery mildew/downy mildew/rust. A high disease pressure was observed in powdery mildew and rust but a moderate level of downy mildew severity was noticed (20-26%). Among the lines screened, VRP-343, VRP-393, NO-17, VRP-147, NO-110 and VRP-233 showed resistance to powdery mildew and rust while VRP-343 and VRP-147 recorded resistance to all three diseases.

On the basis of two year field study, a set of different lines resistance to powdery mildew, rust and downy mildew along with suitable susceptible checks were tested under artificial inoculation conditions. For artificial inoculation, the spores of all the diseases were collected from pea field in the last week of February and sprayed (approx. 10^6 spores/ml) on all the germplasm lines uniformly in polyhouse. The genotypes VRP-343 and VRP-147 were found resistant for all the three diseases whiles susceptible check showed 100 per cent disease incidence and the results were consistent while comparing previous experiments.

Morpho-agronomic characteristics: VRP-343 is a late maturing genotype. It takes 59-60 days for 50% flowering. Flowering starts after 13.2 nodes. Pod length is 6.10 cm and width is 1.36 cm. Pods are green in colour and slightly curved. Number of pods/plant is 26.4 and 10 pod weight is 42 g and each pod has 6.5 seed. Yield/plant is 110g and shelling percent is around 46.5. The total sugar is 3.15 %.

17. Pune Selection-1 (PS-1) (IC0611690; INGR15030), a Papaya (*Carica papaya* L.) Germplasm with Field Tolerance to *Papaya Ringspot Virus* and Yellow Pulp Colour

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Pune Selection-1 (PS-1) is a near homozygous, yellow fleshed, dioecious papaya (*Carica papaya* L.) line. The line is consistently showing field tolerance to *Papaya ringspot virus* strain papaya (PRSV-P) with good yield (ICAR Report, 2012; AICRP Annual Report, 2013; Datar *et al*., 2013 and Sharma *et al*., 2011). The parental material was a segregating land race of papaya named Madhubala. PS-1 was selected from the segregating population in 2009. Since then, it is developed into a line by sib-mating and selection at IARI, Regional Station, Pune.

Morpho-agronomic characteristics: Mean height of the plant is 1.85 meters, mean canopy E-W is 2.45 and N-S 2.25 meters, mean stem girth is 14 cm, leaf shape is palmate type, petiole colour is green. Average time required to flower is 59 days. Petal colour is cream yellow. Average length of the fruiting column is 0.70 meters. Mean number of fruits per plant was 35. Mean fruit weight is1336 g. Average yield/plant is 46 Kg. Shape of the fruit is oblong with slight nipple at stylar-end. Mean length of fruit is 24 cm while breadth is 16.5 cm. Flesh colour is yellow (occasionally orange) with average TSS value of 9.3^0 Brix. The line performed better than both the checks (*C. papaya* cv. Red Lady and Pusa Nanha) under severe PRSV-P pressure.

Associated characters and cultivation practices: PS-1 is having field tolerance to PRSV-P. It shows late and mild PRSV infection. Growth, fruiting characters and incidence of PRSV-P in PS-1 along with two checks of papaya, namely, Red Lady and Pusa Nanha are given in Table 1. Under Pune conditions, it is recommended to plant seedlings having six to eight leaves in spring season (February-March). Since the virus transmitting aphid-vector population is minimal from February to June. Seedlings should be raised in insect-proof polyhouse.

Table 1. Comparison of growth, fruiting characters, yield and PRSV reaction of Pune Selection-1 with local checks (Red Lady and Pusa Nanha)

Variety	PS-1					Red Lady					Pusa Nanha				
Characters	2010-11	2011-12	2012-13	2013-14	Ave.	2010-11	2011-12	2012-13	2013-14	Ave.	2010-11	2011-12	2012-13	2013-14	Ave.
Plant Height (m)	1.88	1.92	1.80	1.78	1.85	1.47	1.60	1.58	1.57	1.56	1.48	1.44	1.25	1.19	1.34
Collar Diameter (cm)	14	14	14	14	14	12	14	14	14	14	13	13	12	12	13
Fruiting Height (cm)	64	75	81	79	75	71	77	81	79	77	56	52	52	46	52
Fruiting Column Length (cm)	84	89	54	51	70	71	62	54	51	60	70	68	47	53	60
Yield (Kg/Pl)	46.9	48.1	40.5	50.1	46.4	18.7	19.2	17.5	13.7	17.3	40.7	39.5	26.4	39.9	36.6
Average Fruit Weight (g)	1283	1366	1361	1334	1336	818	971	948	746	871	1080	1038	1151	1070	1085
Flesh Thickness (cm)	2.72	2.51	3.53	3.00	2.94	2.50	2.88	3.25	2.53	2.79	3.50	3.85	3.75	2.63	3.43
Flesh Colour			Yellow					Red					Yellow		
TSS (^{0}Brix)	9.1	8.4	10.6	9.0	9.3	8.04	8.0	7.5	9.0	8.14	7.5	8.0	7.5	7.5	7.63
PRSV Infection (%)	47.2	44.6	45.5	42.7	45.0	92.3	89.6	84.5	92.6	89.8	62.3	68.3	63.3	61.5	63.8

Being a dioecious line, two seedlings per hill should be planted to maintain higher ratio of productive female plants. Per plant space required is 4.3 to 4.4 square meters that can be achieved by maintaining row to row and plant to plant distance of 2.4m x 1.8 m respectively or by square plantation of 2.1 m x 2.1 m. About 2,300 plants can be accommodated per hectare under both spacings. One square foot FYM, 2 Kg neem seed cake and 1 Kg sterameal should be applied per hill before plantation. Inorganic fertilizers N:P:K at the rate of 300:300:300 g/plant should be applied in four split doses at alternate month. Foliar application of a balance mix of all micronutrients (2g/l) at alternate month along with additional spray of boron (2g/l) at the time of fruit setting, and calcium (2g/l) before fruit ripening is recommended.

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18. IG 91-1100 (IC0612055; INGR15031), a Sugarcane (*Saccharum* Spp.hybrid) Germplasm with Good Seed Setting

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The narrow genetic base of the sugarcane varieties currently under cultivation has posed serious limitations in breaking yield. Past few decades, considerable attention is being given to broaden the genetic base using wild species related to *Saccharum*. The clone IG 91-1100 is polycross hybrid between CoC 772 and *Erianthus* spp which was developed at Sugarcane Breeding Institute, Coimbatore. It was evaluated over the years and identified as one of the potential parents for developing sugarcane varieties with high yield, quality and stress tolerance with good seed set.

Seventeen high yielding clones derived from IG 91-1100 were accepted for pre zonal varietal testing and seven clones were given Co status. Two sugarcane varieties viz., Co 06027 and Co 06030 derived from IG 91-1100 were notified for commercial cultivation under Peninsular and East coast zone respectively during the year 2013. One clone Co 2010-22 having very high yield potential was identified as a biomass type. In addition to high yield and quality, it has also imparted stress tolerance in many Co canes indicating its potential. It has very erect robust canes, arched leaves and tight clasping leaf sheath. Internodes have heavy wax and big pentagonal buds. It is a regular flowerer with 65 % pollen fertility.

Molecular fingerprinting of 13 recently notified sugarcane varieties for commercial cultivation for Peninsular zone and East coast zone using STMS markers indicated, two varieties *viz.*, Co 06027 and Co 06030 developed by crossing CoC 671 and IG 91-1100 were more diverse than the other varieties (Hemaprabha *et al.*, 2013). The clone IG 91-1100 has produced more seedlings in the crosses compared to other intergenic hybrids involving *Erianthus* and can be used to obtain more seedlings in further crosses. Hence, this clone IG 91-1100 can be used as a potential parent to diversify the sugarcane genetic base as well as sustaining sugarcane productivity.

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Hemaprabha G, PJ Priji, TS Sarath Padmanabhan (2013) Molecular fingerprinting of recently notified sugarcane (*Saccharum* L.) varieties using STMS markers. *Journal of Sugarcane Research.* **3**: 107-117.

19. GU04(28) EO-2 (IC0612056; INGR15032), a Sugarcane (*Saccharum* Spp. hybrid) Germplasm with Regular Flowering, Pollen Fertility of 5.5%

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The narrow genetic base of sugarcane varieties has imposed serious limitations in making a significant improvement in sugarcane productivity. In view of this, attempts are being made at all cane breeding stations to broaden and diversify the genetic base of sugarcane through the introgression of wild relatives. Of late, considerable attention is being given to use *Erianthus* spp. which had high biomass potential and high tolerance to biotic and abiotic stresses. GU04(28)EO-2 is a unique and true intergeneric hybrid between *Erianthus procerus* 'IND 90-776' × *Saccharum officinarum* 'PIO 96-435' which was developed at Sugarcane Breeding Institute, Coimbatore during 2004. It is having intermediate juice brix of 13.92% and sucrose of 8.20%. Evaluation under both drought and normal conditions during 2013-14 had shown that it is relatively drought tolerant.

The true hybridity of clone GU04(28)EO-2 has been confirmed through microsatellite primers and 5srDNA primers. The hybrid had both the fragments of *Saccharum* and *Erianthus*. The plant is morphologically distinct and has erect growth habit with dark green leaves without splits in the internodes. The colour of leaf sheath is purple with medium waxiness without spines. It has unique deltoid ligule with hairiness which is present in *Erianthus* Spp. It has big pentagonal bud and swollen root eyes. It is tolerant to drought and also moderately resistant to red rot. It is a regular flowerer with low pollen fertility of 5.5% and can be used as safe female for introgression. It has *Erianthus* cytoplasm and could serve as a genetic stock for diversifying the genetic base of sugarcane and developing drought tolerant cultivars.

20. GU04 (50) RE-16 (IC0612058; INGR15033), a Sugarcane (*Saccharum robustum* × *Erianthus* hybrid) Germplasm with Regular Flowering and Good Seed Set (128 Seedlings)

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Traditionally *Saccharum spontaneum* had been the donor for many important traits in sugarcane. In recent years, considerable attention is given to use *Erianthus* spp. which had been identified as valuable source for many traits such as ratoonability, tolerance to environmental stresses, vigour, disease resistance and potential source for developing energy canes. (Piperidis *et al.*, 2000). Among the different species, *Erianthus arundinaceus* (Rez.) Jeswiet is of specific interest because of the presence of vegetative canes and broad leaves. GU04(50) RE-16 is a true and novel intergeneric hybrid between improved *Saccharum robustum* PIR 00-1100 and *Erianthus arundinaceus* IK 76-91 which was developed at Sugarcane Breeding Institute, Coimbatore during 2004. This is the first report about the production of intergeneric hybrids between two wild relatives *viz*., *S. robustum* and *E. arundinaceus*. It was evaluated for yield and quality traits during 2006-2010. It recorded a mean sucrose of 12.98% with medium purity (74.26%) The true hybridity has been confirmed through cytologically and using *Erianthus* specific markers. It had a somatic chromosome number of 2n=80 with n+n transmission. The hybrid has semi erect stools with thin cylindrical internodes and heavy wax. It has two rows of root eyes with pentagonal

bud without bud cushion. It has narrow dark green leaves with prominent reddish dewlap. Evaluation of the hybrid GU 04(50) RE-16 for flowering and seed set during 2007-2013 showed that it is a regular flowerer and has produced better seed set compared to other intergeneric hybrids. It can be a donor for genes of value from the wild relatives *Erianthus* and *S. robustum*.

Reference

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21. GU08 SSH-66 (IC0612060; INGR15034), a Sugarcane (*Saccharum* × *Sorghum* hybrid) Germplasm with High Early Sugar Accumulation

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Intergeneric hybrids between *Saccharum* and Sorghum were produced as early as 1929 by Venkatraman to develop early maturing sugarcane varieties. But it was not successful. Four intergeneric hybrids with sorghum cytoplasm were produced by crossing sorghum as female parent with sugarcane (Nair, 1999). All the hybrids resembled sugarcane but lacked vegetative vigour and sugar. Fresh crosses were made between sugarcane and sweet sorghum at Sugarcane Breeding Institute, Coimbatore during 2008 and generated 24 putative hybrids. Among the 24 putative hybrids, nine of them were confirmed as genuine hybrids using SSR markers including GU 08 SSH 66. The hybrids involving sorghum were evaluated for their early sugar accumulation at sugarcane Breeding Institute, Coimbatore for two years during 2010-2011. The results showed that the hybrid GU 08-SSH-66 recorded the highest HR brix of 20.80% at 6th month followed by GU 08-SSH-68 (19.80%). The sugar accumulation was steadily increased in all the hybrids and the hybrid GU 08-SSH 66 had the maximum of 23.5% at 8th month (Mohanraj *et al.,* 2013)**.** Seven intergeneric hybrids involving sweet sorghum were also evaluated for red rot under controlled condition testing. Out of which GU 08 SSH 66 was identified as moderately resistant to red rot.

The hybrid had erect stool as well as leaf habit with greenish purple canes and heavy wax. It is medium height with cylindrical internodes and corky patches. Leaf sheath is without hairs and brown dewlap. Bud is small, round without bud grove. Since the intergeneric hybrid GU08 SSH 66 had early high sugar accumulation and red rot resistance it could serve as a diverse source for developing early maturing short duration sugarcane varieties.

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22. 99WL-379 (IC0612061; INGR15035), a Sugarcane (*Saccharum* Spp. hybrid) Germplasm with High Juice Quality Under Water Logged Condition

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99 WL 379 is a high sugar clone developed from cross between Co 7313 (Female parent) with high juice quality, and Co 96011 (Male parent) with waterlogging tolerance from the first selection cycle through a simple recurrent selection scheme. The hybrid is distinct from its female parent for cane thickness and from its male parent for high sucrose content. It is characterized by thick (3.1cm girth) semi erect stem with cylindrical and zigzag arranged internodes. The exposed internode is yellowish green in colour, without ivory and weather marks but with a few shallow cracks. The buds are generally small ovate with apical germ pore and bud cushion but without bud groove. It has open semi-drooping canopy with green coloured lamina. The leaf sheath colour is green with light coating of wax, hard spines and medium loose clasping on the stem.

The clone showed significantly higher juice brix, sucrose % and commercial sugar yield per plot compared to control (Table 1) under waterlogged stress. Excess moisture stress/waterlogging stress is one of the major constrains in sugarcane agriculture. About 2.2 Lakh hectares sugarcane is getting affected by waterlogging across the sugarcane growing states in the country (Nair 2012) and this clone can be cultivated under such situation. It also showed high stem nitrogen content (0.493%), a physiological trait highly correlated with waterlogging tolerance with 32% and 24% higher over the standard varieties (Gomathi and Chandran, 2010 and & 2012).

Table 1. Commercial sugar yield and quality traits of 99 WL 379 under waterlogged condition

Character	Average over three years	Percentage improvement over control	
		Co 62175	Co 8231
Juice brix (%)	20.88	10.73	On Par
Sucrose %	18.88	14.6	2.1
Commercial cane sugar (%)	13.3	18%	1.5%
Commercial sugar yield/ plot	5.86	2.1%	20.5%
Purity	84.02	6.9%	On par

The real potential of this genetic stock is as a parental clone for yield and quality. The clone shows moderate flowering with 40 to 50% pollen fertility and hence has the potential to use as both male and female parent. By using as female parent one hybrid clone (WL 05-499) with high juice quality and sugar yield entered in pre-zonal varietal trial, and as a male parent one hybrid clone identified as Co cane (Co 12015) with high juice quality.

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23. IIHRMGYP-1 (IC0613361; INGR15036), a Marigold (*Tagetus erecta* L.) Germplasm with Petaloid Sterile Flowers; Abiltity to be Multiplied by Cuttings

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IIHRMGYP-1 is a petaloid male sterile genotype useful for breeding program. Flowers are male sterile consisting of only ray florets with well developed gynoecioum and are devoid of disc florets. The genotype can be maintained by vegetative propagation through tip cuttings. With high yielding potential, it has the potential for flower production besides being a valuable genetic stock for hybrid seed production.

Marigold (*Tagetes* spp.) is one of the most popular flowering annuals grown for loose flowers, landscape gardening and pot plants. The flowers are suitable for garlands and floral decorations. Marigold is also widely used as an ingredient for nutraceutical, cosmetic, and pharmaceutical applications. In India, Marigold occupies the first position among flower crops in terms of area covered. As per the estimation of NHB data for the year 2013-14, out of the 255.02 thousand ha. area under flower crops, marigold is covered in an area of 55.89 thousand ha.

Though marigold is being widely used in India, there are not many varieties available and F_1 hybrids are being imported from outside the country and are gaining popularity considering their flower quality and production potential. Identification of male sterile lines is important for exploitation of heterosis and hybrid development. Not much information is available on male sterility in marigold. All the information available about male sterility is limited to apetaloid type of sterility where in flower petals are degenerated (He *et al*., 2010; Gupta *et al.*, 2013).

Ongoing research program at Indian Institute of Horticulture Research, Bengaluru is focused on development of male sterile lines as a prerequisite for heterosis breeding. Crosses are attempted between plants from wide genetic back ground to create variability and for introgression of characters. Individual plant selection made in the breeding population has resulted in a pool of genetic stocks varying in morphological and reproductive characters. IIHRMGYP-1 is an individual plant selection from the segregating population resulting from hybridization between MG-87 and MG-32. The parents, MG-87 and MG-32were heterogeneous and heterozygous population and crossing were attempted between selected plants. From the resulting segregating population, promising progeny plants were selected and multiplied by vegetative propagation followed by clonal selection.

Marigold belongs to Asteraceae family characterized by capitulum inflorescence with peripherally located ray florets and disc florets in the centre. For commercial utility, these inflorescences are considered as flowers and ray and disc florets are considered as petals. Flowers of IIHRMGYP-1 are yellow gold in colour with RHS colour chart No. 12-A in Yellow Group. Distinct features of the genetic stock are its double flowers that are sterile. Sterile flower devoid of petals with all the petals converted to stigma was reported in marigold (He *et al.*, 2010). On the contrary, flowers of novel genetic stock IIHRMGYP-1 developed at IIHR is a petaloid male sterile with double flower type filled with ray florets and are devoid of disc forets and androecium. Plants are of medium height (70 cm), with spreading plant habit and strong branching. All the reported variability in marigold are related to quantitative characters and not much information is available on variability available in sterile types (Singh and Misra 2008; Yuvraj and Dhatt 2014).

In general, marigold varieties are multiplied by seeds and are also amenable for tissue culture (Gupta *et al.*, 2013). We have observed IIHRMGYP-1 having the ability to be propagated by vegetative propagation. We were able to establish a population of IIHRMGYP-1 by tip cuttings and production potential was estimated in replicated trials in comparison with check varieties.

Performance evaluation of IIHRMGYP-1, over three years in replicated trials has revealed it to be having higher production potential compared to the check varieties that includes pure lines of IIHR (IIHRMY4 and IIHRMY5), a high yielding variety 'Pusa Basanthi' and a commercial

Table 1. Production evaluation of IIHRMGYP-1 in comparison with check genotypes

Genotypes	No of flowers/plant	Flower wt (gms/flower)	Flower yield/ plant (gms)
IIHRMYP-1	150.8	6.0	904.8
IIHRMY 4	125.8	5.5	691.9
IIHRMy 5	127.6	5.4	689.0
Pusa Basanthi	119.8	5.2	623.0
F1 Hybrid Gold	118.4	6.4	757.8
c.d@5%	4.3	0.3	249.1
C.V %	6.1	2.9	8.4

hybrid variety 'F_1 Hybrid Gold' (Table 1). IIHRMGYP-1 has the potential for commercial flower production and with its sterile flowers can also be exploited for hybrid seed production.

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