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Assessment of Genetic Divergence in *Brassica* Germplasm

SK Sandhu* and VP Gupta**
Department of Genetics, Punjab Agricultural University, Ludhiana-141004, Punjab

The objective of this study was to assess the genetic divergence available in *Brassica* germplasm based on Euclidean distances for the identification of genetically diverse and agronomically superior accessions from different *Brassica* species which may generate putative transgressive segregates on hybridization. Based on results obtained in field experiments, designed in compact family block, for two consecutive years, wide physio–morphological divergence were obtained for all the traits under study, both within and between species (though the inter-specific variation was higher than intra-specific one). Principal component analysis, which transformed all the metric traits into single index of similarity, yielded eight eigen vector and eigen roots. Based on first five principal components (which accounted for 97.83% of the total variation), non-heirarchical Euclidean cluster analysis grouped the 36 *Brassica* genotypes into five well characterized groups (based on aggregate effect of similarity in traits). Genotypes of each *Brassica* species grouped separately, depicting characteristic differences among species.

Key Words: *Brassica*, Cluster Analysis, Divergence, Germplasm

Rapeseed mustard crops account for almost 14% of the edible vegetable oil supply of the world. Inspite of the competition from soybean and palmolive oils, the rapeseed mustard oil is expected to retain its current share in the international trade. The importance of oil seeds in Indian agricultural economy is well recognized. The oil seed productivity has increased almost three times since 1950-51 through inter-specific hybridization among desirable parents. Systematic improvement in any crop species depends upon the variability available in the germplasm. Numerous species have been recognized in the genus *Brassica*. *Brassica juncea* (L.) *Czern* and Coss (*raya*; 2n = 36) is the most commonly cultivated species with high productivity and greater resistance to insects and diseases. *B. napus* (*ghobi sarson*; 2n = 38) has high potential productivity, resistance to white rust and higher oil content. *B. carinata* (Ethiopian mustard; 2n = 34), with a high seed yield potential, has particular promise as an oil crop under dry land conditions. In order to further upgrade the yield potential of these crops and to impart resistance to biotic and abiotic stresses, it is imperative to introduce desirable attributes in a good agronomic base. For this, an inevitable requirement is to genetically characterize the available variability in the germplasm.

The ordination techniques like principal component analysis followed by cluster analysis was found to be useful tool for getting multi-correlated variables into another set of uncorrelated variables which can be utilized for classification of genotypes into homogenous groups. Hence, the genotypes from diverse clusters can be earmarked for different objectives. The use of non-hierarchical Euclidean cluster analysis to estimate genetic divergence and for classification of germplasm is suggested (Arunachalam, 1981). In view of the above, the present studies were taken up with 36 genotypes of *Brassica* to (i) assess within and between variability in the germplasm based on morphological and yield attributes and (ii) perform natural classification in the germplasm accessions for their characterization based on cluster analysis for identification of genetically diverse and agronomically superior genotypes.

Materials and Methods

Twelve *Brassica juncea* genotypes *viz.*, Kranti, Prakash, Pusa-bold, RH-765, RH-8304, RLC-1359, RLM-29, RLM -198, RLM-185, RLM-234, RLM-619 and Varuna; 12 genotpyes of *B. napus* namely, ABU, NB-215, BN-225, BN-510, GS-119, GS-124, GS-203, GSL-1, GLS-1501, HN–5-1, K-1 and Marinus, and 12 genotypes of *B. carinata* namely BIC-3, BS-260M, BS-70-1, DIC-2, DIC-3, HC-1, HC-2, HC-6, HC-7, HC-6-7, HC-6-7, HC-S-1 and PC-5 were grown in a compact family block design with three replications over two years. These genotypes were diverse collection of local selections, breeding lines, cultivars and commercial varieties. Recommended cultural practices and plant protection measures were followed to raise the crop. Data were recorded for two years on plant height (measured from the base to the tip of the plant at maturity in cm), number of primary branches (number of branches on the main stem), siliquae/plant. These observations were recorded on five randomly selected plants of each genotype. Other characters *viz.*, 1000-seed weight, seed yield/plant, oil

* Central Potato Research Station, Jalandhar-144003, Punjab
** Rajendra Agricultural University, Pusa, Bihar

yield/plant (4g of oven dried seeds were taken and oil content was measured using NMR spectroscopy and multiplied by respective seed yield to calculate oil yield) and days to 50% flowering of genotypes were recorded on plot basis.

The principal component analysis was carried out to transform the inter-dependent variables into a set of independent variables (Hottling, 1933; Mardia, 1971). These principal component scores were used to compute Euclidean distances based on non-hierarchical cluster analysis through SPAR 1 (Beale, 1969; Spark, 1973). This method characterizes genetic divergence on the basis of similarity and dissimilarity denoted by aggregate effects of agronomic traits under study.

Results and Discussion

The pooled analysis of variance for morphological and quality traits in 36 genotypes showed the existence of distinct genetic differences among the genotypes in respect of all the characters studied (Table 1). The species-wise analysis of variance to assess inter-specific variation for 12 genotypes of each *Brassica* species showed significant differences among the genotypes (Table 2). Perusal of these studies indicated the presence of sufficient variability demonstrating the agronomic potential in crop *Brassicas*, which could be exploited for further improvement programme. Presence of variability for physio-morphological traits in *Brassica* species (Sandhu and Gupta, 1996) and in *Gobhi sarson* (*B. napus*) for

Table 1. Pooled analysis of variance for morphological and quality traits in *Brassica* germplasm

Source	df	Mean squares							
		Plant height	Primary branch number	Siliqua number	Siliqua length	1000 seed weight	Days to 50% flowering	Seed yield	Oil yield
Genotype	35	2077**	14.2**	4824**	4.18**	1.27**	780**	14.02**	
Error	70	31.5	2.23	34.5	0.23	0.19	6.26	1.76	0.39

** Significant at 1%

Table 2. Species-wise analysis variance for morphological and quality traits in *Brassica* species

Brassica juncea

Source	df	Mean squares							
		Plant height	Primary branch number	Siliqua number	Siliqua length	1000 seed weight	Days to 50% flowering	Seed yield	Oil yield
Genotype	11	4827**	4.13**	881**	0.48**	1.09**	99.1**	17.95**	2.99**
Error	22	45.5	0.57	30.3	0.09	0.19	7.22	2.85	0.72

** Significant at 1%

Brassica napus

Source	df	Mean squares							
		Plant height	Primary branch number	Siliqua number	Siliqua length	1000 seed weight	Days to 50% flowering	Seed yield	Oil yield
Genotype	11	532**	15.9**	1881**	4.18**	1.94**	0.796**	780.29**	14.02**
Error	22	14.6	3.30	20.3	0.23	0.55	0.26	1.76	0.39

** Significant at 1%

Brassica carinata

Source	df	Mean squares							
		Plant height	Primary branch number	Siliqua number	Siliqua length	1000 seed weight	Days to 50% flowering	Seed yield	Oil yield
Genotype	11	934**	13.6**	7369**	0.76	1.86**	85.3**	14.8**	2.09**
Error	22	28.5	2.46	50.6	0.51	0.11	3.06	0.88	0.15

*, ** significant at 1 and 5 % level, respectively

seed and oil yield is assessed in previous studies (Singh *et al.*, 1991)

The mean, range and heritability (in broad sense) estimates for different characters are given in Table 3. A wide range was observed for all the traits except 1000-seed weight where value range from 2.1 to 4.3 g. The heritability was high for seed yield (95.5%), oil yield (94.5%), plant height (92.7%) and days to 50% flowering (98.3%). This suggests that these traits are least affected by the environment and, therefore, can be effectively used as selection criteria. High variability for these traits has also been reported in Swede rape (Li and Guan, 1981).

Correlation matrix was used to transform all the metric traits into a single index of similarity in the form of principal components, which yielded eight Eigen vectors and Eigen roots. The per cent variation explained by eight roots, 1 to 8, was 46.34, 26,37, 15.09, 6.51, 3.53, 1.08, 0.96 and 0.13, respectively. Further, maximum eigen root value was 3.707 obtained by eigen vector 1, followed by 2.110, 1,207, 0.520, 0.283, 0.086. 0.007 and 0.010, respectively, for rest of the vectors. The first five principal components calculated by using standard variables accounted for 97.84% of total variation, hence used for non-hierarchical cluster analysis. The first component was measure of primary branch number, second of seed yield/plant, third of siliqua length, fourth of 1000-seed weight and fifth of plant height and days of 50% flowering, as the coefficients associated with these traits have the largest magnitude (Table 4). The first five principal components were used for cluster analysis. Based on this, 36 accessions were grouped into five well-characterized group (I-V) based on similarity in traits (Table 5). The maximum number of genotypes (12) fell in cluster I, while minimum number (3) was observed in cluster II. This grouping indicated considerable diversity in the germplasm. The important finding in this regard is that all the 12 *B. napus* genotypes gathered into Cluster I, *B. juncea* genotypes converged to cluster II (3) and cluster IV (9) whereas *B. carinata* genotypes grouped into cluster III (5) and cluster V (7). This demonstrates the presence of characteristic differences

Table 3. Estimate of variability parameters for quantitative traits of *Brassica* species

Characters	Mean	Range	C.V. (%)	Heritability*
Plant height (cm)	161.45	110.8 -224.2	15.88	92.7
Primary branch number (cm)	8.21	3.31 - 13.4	26.65	72.7
Siliqua length (cm)	5.25	3.56 - 8.2	22.52	89.6
Siliqua number	138.05	71.6 -195.8	25.89	88.5
1000-seed weight (g)	3.22	2.1 - 4.0	16.62	73.6
Days to 50% flowering	101.23	62.3 -132.9	23.34	98.3
Seed yield (g)	12.49	5.26 - 21.4	38.19	95.5
Oil yield (g)	5.13	1.33 -10.26	45.78	94.5

*Heritability in broad sense

Table 4. Eigen vectors for eight standardized variables for the first five principles

Variables	Principal				
	1	2	3	4	5
1	0.0470	-0.011	-0.037	-0.144	0.493
2	0.0628	-0.612	-0.384	-0.233	0.104
3	-0.0110	-0.123	-0.673	0.713	0.009
4	0.1300	-0.378	0.614	0.48	0.182
5	-0.7150	0.645	-0.093	-0.034	0.098
6	-0.1960	-0.217	-0.242	-0.138	0.684
7	-0.1810	0.0840	-0.300	-0.151	-0.177
8	-0.0620	0.0000	-0.072	0.032	-0.091

1-Plant height, 2-Primary branch number, 3-Siliqua length, 4-1000-seed weight, 5-siliqua number, 6-Days t 50% flowering, 7-seed yield.

among *Brassica* species for morphological and yield traits which could be exploited for improvement programme. Inter-and intra-cluster distances, which provided index of genetic diversity among and within the clusters, revealed that intra-cluster distances were of lower magnitude as compared to inter-cluster distances. It suggested that genotypes of same cluster had little divergence from each other. Therefore, the hybridization among the accessions of same cluster is not desirable. The intra-cluster distance ranged from 1.025–1.835. The minimum inter-cluster distance was observed between II and IV (2.984) followed by II and V (2.992). Maximum inter-cluster distance was between IV and V (5.050) followed by II and III (5.017) (Table 6). The crosses between the genotypes from the clusters may give putative transgressive segregates. It is suggested that better recombinants can be obtained by selecting genotypes with their phenotypic dissimilarity (Sharma *et al.*, 1998).

Cluster-wise mean values for each character under study are given in Table 7. The cluster IV genotypes (*B. juncea*) were characterized with high seed yield and oil yield. *B. carinata* genotypes (Cluster V & VII) had the highest plant height and primary branch number but they are late maturing (as indicated by high values for days of 50% flowering) and also the poorest yielders with lowest oil yield. The screening of germplasm helped in identifying promising genetic stocks, on the basis of their mean performance for important physio-mprphological traits and genetic divergence (Table 8). Use of these genotypes in *Brassica* breeding programme is suggested as multiple crossing among them may result in segregates with commercially desirable attributes. A similar approach was also followed for large collections of wheat (Garg and Gautam, 1997), and maize (Katiyar *et al.*, 1998) germplasm for selection of diverse and superior genotypes.

Table 5. Distribution of genotypes of different clusters

Cluster No.	No. of genotypes	Name of genotypes
I	12	GSL-1, GSL-1501, ABU, BN-215, Marinus, GS-119, BN-225, K-1, GS-203, HN-5-1, NH-510 and GS-124
II	03	Pusa-bold, RLC –1359 and RH-765
III	05	HC-1, DIC-2, PC-5, DIC-3 and HC-6
IV	09	Varuna, HR 8304, RLM-29, Prakash, RLM-198, Kranti, RLM-619, RLM-185 and RLM-234
V	07	HC-2, HC-7, BIC-3, HC-6-7, BS-26-M, HC-S-1, and BS-70-1.

Table 6. Estimates of average intra- and inter-cluster distance

Cluster number	I	II	III	IV	V
I	**1.835**	3.485	3.761	3.882	2.901
II		**1.025**	5.017	2.984	4.053
III			**1.298**	4.214	2.992
IV				**1.113**	5.051
V					**1.064**

Diagonal (bold face) values are average intra-cluster distances and under lined values are the highest (5.051) and the lowest (2.901) inter-cluster distances.

Table 7. Average performance of different clusters for morphological, yield and yield contributing traits

Cluster number	No. of genotypes	Plant height (cm)	Primary branch number	Siliqua number (cm)	Siliqua length	1000-seed weight	Days to 50% flowering	Seed yield (g)	Oil yield (g)
I	12	144.35	7.25	6.67	3.12	121.18	11.5.16	11.17	4.67
II	3	1234.93	6.32	4.28	3.94	160.93	73.4	14.73	6.11
III	5	201.76	11.74	4.29	2.77	137.18	112.58	9.87	3.63
IV	9	168.17	8.06	4.92	3.01	182.76	66.86	19.26	8.23
V	7	169.01	8.35	4.36	3.69	100.33	125.36	6.97	2.44

Table 8. Important genetic donors identified from germplasm for different characters

Plant height (>182cm)	HC-1, HC-2, DIC-2, DIC-3, PC-5
Primary branch number (>9.6cm)	HC-1, HC-6, HC-7, DIC-2, DIC-3, PC-5
Siliqua length (>6.67 cm)	ABU, BN-215, GS-2043, GSL-1501, HN-5-1
1000-seed weight (>3.30g)	Pusa bold, RH-765, RLC-1359, RLM-29, Varuna
Siliqua number (>170)	Prakash, RH-765, RH-8304, RLM-29, RLM-185, RLM-198, RLM-234, Varuna
Days to 50% floweimg (≤63)	Kranti, RLM-234, Varuna
Seed yield (>18.0g)	RH-8304, RLM-29, RLM-185, RLM-234, Varuna
Oil yield (>8.00g)	RH-8304, RLM-29, RLM-185, RLM-234, Varuna

Conclusions

The study depicted the relative divergence in morphological and quality traits. The screening of genotypes helped in identifying the promising genotypes for different traits, which may serve as good genetic donors for exploitation in further breeding programme. The genotypes occupying the top position in superior clusters may further be assessed for their combining ability and gene effects following suitable mating designs.

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Adaptability of Exotic Potato Germplasm in North-Western Hills of India

J Gopal
Division of Crop Improvement, Central Potato Research Institute, Shimla-171001, Himachal Pradesh

Late blight resistance and high tuber yield are the two major requirements of potato crop in North-western hills of India. Adaptability of 176 exotic germplasm accessions from 18 countries was studied under these conditions for 2-4 years against Kufri Jyoti, the most popular variety of the region. Most of the accessions were inferior or at par with Kufri Jyoti for one or the other character. There was no relationship between resistance to late blight and performance for tuber yield and its components. Ten accessions were resistant to late blight, 41 moderately resistant and the remaining were found susceptible. Eight accessions were identified as promising being resistant or moderately resistant to late blight and having high tuber yield with acceptable tuber size, shape, eye depth. Strategies for exploiting the various accessions in potato breeding programmes are discussed.

Key Words: Adaptability, Exotic Germplasm, Late Blight, Potato, *Solanum tuberosum*

Potato (*Solanum tuberosum* L.) is believed to have been introduced in India from Europe towards the beginning of the 17th Century (Pushkarnath, 1964). A systematic research on potato in India was started in 1949 with the establishment of Central Potato Research Institute (CPRI), Patna (headquarters shifted to Shimla in 1956). Development of improved potato varieties for different agro-climatic regions of India, has been the major mandate of CPRI since its establishment. To achieve this objective, acquisition of exotic potato germplasm from different countries has been a continuing activity of CPRI. Germplasm accessions so obtained are evaluated for various biotic and abiotic factors to identify parents suitable for use in potato breeding programmes of the country. In North-western hills of India, potato is grown under temperate long-day conditions of summer (April/ May to August/September). Late blight caused by the oomycete *Phytophthora infestans* is a serious problem in this region, and under epiphytotic conditions yield loss can be as high as 90%. In fact this is the most destructive disease of potatoes worldwide. Resistance to late blight and high tuber yield are thus the major requirements of potato crop in North-Western hills of India. The present paper reports adaptability of 176 exotic germplasm accessions received from 28 countries, under these conditions.

Materials and Methods

One hundred and seventy six accessions available in the germplasm repository of CPRI, were divided into three sets (Tables 3-5) depending upon the number of tubers available in various accessions. These were evaluated at the Central Potato Research Station, Kufri (37° N, 77° E, 2500 m above sea level) in two types of trials *i.e.* non-replicated row trial in which an accession was represented by 1-2 rows of 10 tubers each; and replicated randomized complete block design trials with 2-3 replications and a plot size of 3 rows of 10 tubers each.

Set 1: In this 20 accessions were evaluated for four years, in row trials for initial two years and in replicated trials for the remaining two years.

Set 2: In this 56 accessions were evaluated for three years, in row trials for initial two years and in a replicated trial in the final year.

Set 3: In this 100 accessions were evaluated for two years in row trials.

Kufri Jyoti the most popular variety of the region, was used as check in all the trials. This variety being susceptible to late blight also acted as spreader of the disease and for this it was planted after every 10-15 rows (Dowley *et al.*, 1999). Seed tubers of all accessions and check variety were produced at Kufri (a seed production site) and had received the same post-harvest and storage treatments. Genotypes were randomized in each year. All trials were planted at intra-and inter-row spacing of 50 and 20 cm respectively. Normal cultural schedules were followed. However, no fungicide was applied for late blight control in order to screen the accessions against this disease (Dowley *et al.*, 1999). No artificial inoculation was done for this disease as in this region potato is severely attacked by the late blight every year and the objective was to assess the accessions for adaptability under natural conditions.

Data on late blight incidence were recorded in all the trials when the crop was 90 days old and the check variety Kufri Jyoti had been completely killed by this disease. Accessions were grouped into three classes based on data averaged over the years: Resistant (foliage damage < 20%), Moderately Resistant (foliage damage 20-50%) and Susceptible (Foliage damage > 50%). Haulms were cut when the crop was 100 days old (the maximum acceptable crop duration of this region) and at harvest, data were recorded on plot basis for tuber yield (g/plant), tuber number/plant and average tuber weight (g). Kufri Jyoti was planted as check variety after every 10-15 rows and its mean performance was used in the analysis.

Standard statistical procedure was followed by analyzing data of replicated trials. Pooled analysis over the years was done using non-replicated and replicated data (mean over replications) to partition the observed variation into year, genotype and residual components. Since accessions were grouped into three qualitative grades for late blight reaction, no analysis was conducted for this character.

Results

Set 1: Analyses of variance showed that tuber yield as well as its components were affected by year (Table 1a) whereas replication effect within a year was non-significant for all characters (Table 2a). Average performance over four years showed that all accessions were at par with the check variety Kufri Jyoti for tuber yield and average tuber weight. Two accessions (CP 2370 and CP 2380) had more number of tubers than Kufri Jyoti. Three accessions namely CP 2370, CP2384 and CP 2385 were resistant to late blight, seven were moderately resistant and remaining were found susceptible (Table 3).

Set 2 : Like set 1, replication effect was non-significant in this set also (Table 2b). Year effect was significant only for tuber number (Table 1b). Average performance over three years showed that accessions CP Nos. 1690, 2415, 3098, 3127 and 3157 yielded higher than the check. These genotypes performed better than Kufri Jyoti in the replicated trial also. Eight accessions had more tubers and three had higher average tuber weight than Kufri Jyoti. In this set, only one accession CP 3147 was resistant to late blight, 10 were moderately resistant and remaining susceptible (Table 4).

Set 3: In this set, year effect was significant for tuber yield but non-significant for yield components (Table 1c). Average performance pooled over two years showed that only four accessions CP Nos. 1673, 2110, 2378 and 3250 yielded higher than Kufri Jyoti, whereas 7 accessions had more tubers than Kufri Jyoti. Only three accessions (CP 2332, CP2940 and CP3250) had higher average tuber weight than Kufri Jyoti. Six accessions were resistant to late blight, 25 were moderately resistant and remaining were susceptible (Table 5).

Table 1. Analysis of variance for performance of accessions for tuber yield and its components over years

a. Set 1

Source	df	Mean squares		
		Tuber yield	Tuber number	Av. Tuber weight
Year	3	124233**	94.72**	2410.91**
Accession	20	7035	9.63**	377.35
Residual	60	8173	3.99	244.59

b. Set 2

Source	df	Mean Squares		
		Tuber yield	Tuber number	Av. Tuber weight
Year	2	2076	17.08*	24.20
Accession	56	31808**	15.93**	1025.53**
Residual	112	7075	5.13	154.07

c. Set 3

Source	df	Mean Squares		
		Tuber yield	Tuber number	Av. Tuber weight
Year	1	46264*	7.45	500.61
Accession	100	27176**	15.83**	618.52**
Residual	100	8307	7.34	204.95

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

Table 2. Analysis of variance for performance of accessions for tuber yield and its components in a year

a. Set 1

Source	df	Mean squares					
		Ist Year			2nd Year		
		Tuber Yield	Tuber number	Av. Tuber weight	Tuber yield	Tuber number	Av. Tuber weight
Replication	2	6247	1.19	3.08	159	1.39	69.89
Accession	20	27204**	12.13**	535.44**	8439**	5.67**	296.20**
Residual	40	2960	1.70	72.72	1076	0.60	59.65

b. Set 2

Source	df	Mean Squares		
		Tuber yield	Tuber number	Av. Tuber weight
Replication	1	358	0.52	140.98
Accession	56	41828**	15.84**	1203.74**
Error	56	1405	3.35	109.05

** Significant at 1%

Table 3. Mean performance of 20 exotic germplasm accessions over four years

CPRI Accession number	Donor's culture/variety name or number	Source country	Tuber yield (g/plant)	Tuber number per plant	Av. Tuber weight (g)	Reaction to late blight
CP2278	Maris Peer	Bangladesh	234	5.2	45.9	S
CP2292	P-3	Peru	188	3.9	51.6	S
CP2297	P-6	Peru	305	4.0	79.4	MR
CP2333	AA-624	Ethiopia	314	8.2	37.2	MR
CP2334	AL-575	Ethiopia	273	5.4	52.5	MR
CP2335	Ica Sirena	USA	245	5.9	45.8	S
CP2348	Stina	Sweden	205	5.4	38.9	S
CP2358	Islander	USA	292	8.1	39.4	S
CP2370	Muziranzara	Peru	237	8.4	33.1	R
CP2371	LT-8	Peru	261	6.3	43.9	S
CP2380	CFQ69.1	Mexico	319	10.3	36.2	MR
CP2381	CFJ69.1	Mexico	353	7.9	47.6	MR
CP2384	AGG69.1	Mexico	298	7.8	45.9	R
CP2385	AND69.1	Mexico	258	6.2	42.4	R
CP2390	BL1.5	UK	273	6.4	38.3	MR
CP2407	Montsama	Mexico	297	7.7	40.1	MR
CP2420	Mineira	Brazil	242	6.8	35.6	S
CP2421	Serrana Inta	Argentina	268	7.1	42.2	S
CP2427	Pirola	Germany	227	5.6	43.0	S
CP2428	Granola	Germany	323	6.6	51.2	S
Check	K. Jyoti	India	258	5.5	50.1	S
	LSD (5%)		n.s	2.8	n.s.	

R = resistant; MR = moderately resistant; S = susceptible

Table 4. Mean performance of 56 exotic germplasm accessions (set 2) over three years

CP No.	Donor's culture/variety	Source country	Tuber yield (g/plant)	Tuber number per plant	Av. Tuber weight (g)	Reaction to late blight
CP1636	Ackersegen	Germany	117	3.9	40.1	S
CP1690	Irish Cobbler	USA	466	7.5	62.2	MR
CP2125	Up-to-date	UK	266	6.5	39.8	S
CP2181	I-931	India	271	8.9	36.6	MR
CP2277	Dohazari Sada	Bangladesh	62	7.2	9.5	S
CP2280	Lal Pakari	Bangladesh	74	8.5	8.1	S
CP2339	Garana	Madagascar	87	7.0	27.9	S
CP2362	Timate	Netherlands	344	5.2	67.3	S
CP2364	Isna	Bulgaria	197	3.7	61.2	S
CP2413	Piratini	Brazil	306	11.7	29.1	S
CP2415	MEX.750821	Mexico	399	10.5	38.6	MR
CP2417	MEX.750838	Mexico	221	9.5	32.9	MR
CP3079	Bintje	Netherlands	230	5.3	42.6	S
CP3084	Baraka	Netherlands	244	4.4	58.0	S
CP3087	Hertha	Netherlands	229	3.8	69.3	S
CP3088	Obelix	Netherlands	313	4.7	66.3	S
CP3089	Origo	Netherlands	205	4.2	46.8	S
CP3098	27/15	Peru	420	6.5	59.3	MR
CP3099	Lal Shil	Bangladesh	27	5.2	5.10	S
CP3100	Nishiyutaka	Japan	167	3.2	58.6	S
CP3101	Esperanza	Ecuador	105	8.3	11.8	S
CP3111	Huaycha	Peru	69	3.5	19.9	S
CP3124	CIP379676.3	Peru	178	8.0	24.1	S
CP3125	SR-1	Peru	253	7.8	33.1	S
CP3126	Haille	Peru	241	5.7	42.4	S
CP3127	CIP378711.2	Peru	423	7.3	59.2	MR
CP3134	Santa Cecilia	Ecuador	89	12.8	7.7	S
CP3142	Maine-28	USA	267	2.8	100.7	S
CP3144	Desital	Italy	160	3.5	48.3	S
CP3145	Yana	Peru	63	3.0	20.2	MR
CP3147	Muruta	Peru	279	10.4	26.8	R
CP3148	Muru	Peru	66	4.6	14.6	MR
CP3151	CIP379693.153	Peru	236	5.3	43.1	S
CP3152	TM-3	Peru	101	3.3	33.3	S
CP3153	TM-4	Peru	103	3.8	26.1	S
CP3154	P110	Peru	242	7.5	34.6	S
CP3156	Monserrate	Colombia	137	9.4	14.1	MR
CP3157	Dalia	Poland	424	5.8	70.9	S
CP3158	Ruta	Poland	322	8.0	41.2	S
CP3159	Lotos	Poland	334	6.6	45.1	S
CP3160	Janeka	Poland	310	5.6	62.3	S
CP3162	Sowa	Poland	252	5.4	47.6	S
CP3165	Tarpan	Poland	270	5.1	56.9	S
CP3166	Pola	Poland	181	3.9	48.7	S
Cp3167	Perkoz	Poland	170	3.6	48.9	S
CP3171	Bzura	Poland	243	6.6	36.8	MR
CP3172	Frezja	Poland	183	4.1	47.5	S
CP3173	Elba	Poland	339	7.4	46.1	S
CP3174	Fala	Poland	190	5.6	36.3	S
CP3175	Stobrawa	Poland	245	6.9	34.2	S
CP3176	Sokol	Poland	308	5.8	51.1	S
CP3178	Elida	Poland	180	4.7	41.1	S
CP3180	Atoe	Poland	248	6.3	38.4	S
CP3193	BW-4	Peru	167	5.8	31.0	S
CP3200	Dejima	Japan	182	3.7	31.0	S
CP3204	MF-II	Peru	156	2.9	49.7	S
Check	Kufri Jyoti	India	239	4.7	48.1	S
	LSD (5%)		136	3.7	20.1	

R = resistant, MR = moderately resistant; S = susceptible

Table 5. Mean performance of 100 exotic germplasm accessions (set 3) over two years

CPRI accession number	Donor's culture/variety name/number	Source country	Tuber yield (g/plant)	Tuber number per plant	Av. tuber weight (g)	Reaction to late blight
CP1673	Dr. McIntosh	Ireland	460	9.5	48.0	MR
CP1998	Michoacan	Mexico	84	4.4	20.5	MR
CP2000	I-1062	India	284	8.4	40.5	R
CP2011	CIP676082	Mexico	434	12.5	42.5	R
CP2013	Atzimbe	Mexico	315	7.0	45.5	S
CP2014	CIP720048	Mexico	286	8.5	54.5	R
CP2053	CIP379376	Peru	175	2.6	65.5	S
CP2110	CFK.69.1	Mexico	488	13.3	36.0	MR
CP2161	Pentland Hawk	UK	394	8.9	44.5	S
CP2272	PI458391	USA	118	4.9	24.0	S
CP2282	Iwa	New Zealand	185	6.2	58.0	S
CP2284	Bremer	Australia	162	4.6	48.5	S
CP2289	Magayar Rozsa	Hungary	211	5.9	35.5	S
CP2293	V-2	Peru	188	6.8	30.0	S
CP2298	P-7	Peru	119	4.9	15.5	S
CP2302	Santo Amor	Brazil	205	5.2	28.0	S
CP2304	Aracy	Brazil	158	4.3	32.0	S
CP2305	Primicia Inta	Argentina	90	4.3	21.0	S
CP2329	KTT60.21.19	Netherlands	333	5.6	60.0	S
CP2330	Atlantic	USA	163	4.5	37.5	S
CP2332	AL-204	Ethiopia	300	4.1	96.5	S
CP2336	Seseni	Zaire	205	6.4	38.5	S
CP2340	104-12-43	Peru	216	3.6	50.0	S
CP2346	F6	Peru	201	5.2	40.0	S
CP2347	Gasore	Peru	263	6.1	40.5	S
CP2351	Tobique	Canada	123	3.6	27.5	S
CP2355	Yankee Supreme	USA	195	5.8	24.5	S
CP2360	Palma	Switzerland	229	3.5	23.0	S
CP2363	Rila	Bulgaria	230	4.7	35.5	S
CP2368	TS-2	Peru	196	4.0	36.5	S
CP2374	TM.1	Peru	204	3.9	28.0	S
CP2376	Cruza-27	Mexico	210	7.2	31.5	MR
CP2377	ABZ69.1	UK	264	8.6	32.0	S
CP2378	Poos.16	Netherlands	453	10.0	44.0	R
CP2379	CEW69.1	Mexico	192	6.3	40.0	R
CP2382	CFS69.1	Mexico	45	4.0	10.5	S
CP2383	AGB69.1	Mexico	229	6.7	35.0	S
CP2391	BL-1.10	UK	190	5.9	39.5	S
CP2392	BL-2.9	UK	171	6.3	27.0	S
CP2397	Santanlalla	Peru	30	3.0	9.0	S
CP2403	Chejchi	Bolivia	238	5.3	46.0	S
CP2409	Yungay	Peru	113	5.5	20.5	S
CP2411	ARX 69.1	UK	221	8.5	25.0	MR
CP2416	MEX 750826	Mexico	315	7.2	37.0	MR
CP2418	Chiquita	Brazil	267	8.6	29.5	S
CP2422	Ballenera	Peru	226	4.7	39.5	S
CP2940	TS-3	Peru	250	2.6	96.5	S
CP3029	Clavela	Chile	88	6.5	13.0	S
CP3072	Flor-Blanca	El Salvador	371	9.4	37.0	MR
CP3080	Alpha	Germany	105	3.4	30.0	S
CP3083	Campbell-14	USA	218	3.9	60.5	S
CP3094	Hybrid-14	Nepal	263	7.7	41.0	S
CP3108	69.56.52	Mexico	114	5.9	21.0	S

Contd.

Table 5. Contd.

CPRI accession number	Donor's culture/variety name/number	Source country	Tuber yield (g/plant)	Tuber number per plant	Av. tuber weight (g)	Reaction to late blight
CP3138	Maria	Euacdor	125	2.8	41.0	S
CP3139	Santa Catalina	Euacdor	42	4.0	9.5	S
CP3163	Irys	Poland	143	5.0	28.5	S
CP3181	G-2	Peru	37	3.7	10.0	S
CP3182	Santaana	Peru	44	2.0	23.0	S
CP3184	Yurac Sinchi	Peru	149	6.9	21.5	S
CP3187	BW-3	Peru	164	4.8	34.5	S
CP3188	Kinga	Peru	212	4.3	49.5	S
CP3189	Sissay	Peru	121	6.2	20.0	S
CP3190	Kinigi	Peru	125	6.3	20.0	S
CP3191	25/40	Peru	140	4.4	25.0	S
CP3197	TS-4	Peru	38	1.9	20.5	S
CP3201	Sanjuan	UK	32	1.7	22.0	S
CP3207	P111	Peru	150	21.3	12.0	MR
CP3208	MF-1	Peru	100	3.6	27.5	S
CP3242	CIP381146.12	Peru	25	4.2	7.0	S
CP3243	CIP381169.16	Peru	291	9.4	29.5	MR
CP3246	CIP381381.13	Peru	275	13.1	21.5	R
CP3248	CIP381376.15	Peru	295	5.1	60.0	MR
CP3250	CIP381379.15	Peru	619	8.9	91.0	MR
CP3250A	Unknown	Peru	200	8.5	24.0	MR
CP3251	CIP381381.20	Peru	364	10.0	36.0	MR
CP3252	CIP381382.34	Peru	175	13.6	43.0	MR
CP3253	CIP381388.34	Peru	53	3.5	15.5	S
CP3254	CIP381403.1	Peru	319	7.9	40.5	S
CP3255	CIP381403.5	Peru	244	6.9	34.5	MR
CP3256	CIP381403.8	Peru	306	7.6	41.0	S
CP3258	CIP382119.17	Peru	44	3.5	10.5	S
CP3259	CIP382119.20A	Peru	275	11.4	24.0	MR
CP3262	CIP382122.23	Peru	125	7.3	17.0	S
CP3263	CIP382138.4	Peru	19	2.3	8.0	S
CP3268	CIP382255.8	Peru	64	6.1	10.0	S
CP3270	CIP383034.8	Peru	88	5.8	14.0	S
CP3272	CIP384298.35	Peru	291	11.2	26.5	S
CP3275	CIP384319.1	Peru	69	3.9	18.0	S
CP3276	CIP384321.3	Peru	238	11.0	20.0	MR
CP3277	CIP384321.9	Peru	253	11.9	19.5	MR
CP3279	CIP384329.21	Peru	48	3.3	11.0	MR
CP3280	CIP384375.3	Peru	370	6.4	22.5	MR
CP3281	CIP381403.22	Peru	342	10.3	33.5	MR
CP3282	CIP382119.27	Peru	15	2.5	5.5	S
CP3283	CIP382150.5	Peru	7	2.5	3.0	S
CP3284	CIP383117.6	Peru	37	2.1	16.5	S
CP3285	CIP384651.14	Peru	125	5.6	20.5	MR
CP3286	CIP385021.12	Peru	148	9.2	16.5	MR
CP3287	CIP386002.4	Peru	200	5.8	34.5	MR
CP3288	CIP384331.10	Peru	172	4.9	23.0	MR
Check	Kufri Jyoti	India	267	6.0	44.0	S
	LSD (5%)		181	5.4	28	

R = resistant; MR = moderately resistant; S = susceptible

Discussion

Genotype x environment interaction is known to be important for tuber yield and its components in potato (Yildrim and Caliskan, 1985; Gopal, 1989) suggesting thereby that location-specific evaluations for such characters should be conducted over the years. In the present study, each accession was tested for a minimum of two years. Replication mean squares were non-significant (Table 2) probably due to high within replication (error) variation on account of soil heterogeneity and micro-climatic variations in the terraces in which the accessions of a set were planted. This is an unavoidable situation due to hilly topography of the region. Birhman and Kang (1993) have also reported non-significant replication mean squares for such characters in potato. In such a situation data based on non-replicated trial should serve the same purpose as the one based on replicated trial. As the clones were tested over years and randomized in each year, reliable estimates of the performance could be obtained. Analysis pooled over the years was conducted by including data from replicated (mean over replications) as well as non-replicated trials. Residual mean squares from such an analysis include both year x accession interaction and error components, and hence LSD values were large (Table 3-5). This was used for testing the significance of year and accession mean squares, as the objective was to identify consistently superior performers.

In potato, susceptibility to late blight is known to be affected by age (Umaerus, 1970): initial phase of high susceptibility is succeeded by an intermediate phase of greater resistance coinciding with the period of most rapid growth, which is followed in turn by a final phase of higher susceptibility as flowering and tuberization begin. To be sure of the resistance to late blight in the accessions tested in the present study, data on late blight incidence were recorded in the final phase of higher susceptibility when the disease was in severe form and the susceptible accessions including Kufri Jyoti had been killed by the pathogen. From a practical point of view accessions were grouped into three grades of resistance, but a continuous variation was observed with regard to disease intensity in various accessions. Highly complex (9-10 genes) races of *Phytophthora infestans* occur at Kufri (Singh and Bhattacharyya, 1999) and there was no chance of escape as the disease was severe in the experimental plots. The resistance observed was thus field resistance controlled by minor genes. Canizares and Forbes (1995) made similar conclusions in their study of the reaction of *Solanum phureja* sub-species *phureja* Juz and Buk to *Phytophthora infestans*. This is the preferred type of resistance as many years of practical experience has shown that race-specific, mostly mono-genically inherited resistance which is expressed as a hyper-sensitive reaction is not durable (Umaerus and Umaerus, 1994). Our results, however, do not exclude the possibility of presence of major genes along with minor genes for resistance to late blight in the accessions tested (Swiezynski *et al.*, 1991). It is rather difficult to separate specific and general resistance to *Phytophthora infestans* (Colon and Budding, 1988: Gees and Hohl, 1988; Turkensteen, 1989).

The results presented in Tables 3-5 showed that there was no relationship between resistance to late blight and performance for tuber yield and its components. Many accessions having more resistance than Kufri Jyoti, were inferior to Kufri Jyoti for tuber yield and its components. A similar situation has also been reported by Swiezynski *et al.* (1991). Such accessions though not suitable for commercial exploitation, can be exploited in breeding programmes to produce progenies with resistance to late blight and some segregants among them may yield higher than Kufri Jyoti as gene action for tuber yield is known to be predominantly non-additive (Gopal, 1998a). Accessions having high tuber yield but susceptible to late blight (*e.g.* CP3157) can also be used in potato breeding programmes by crossing them as females with late blight resistant males as high yielding females may result in high yielding progenies (Gopal, 1998b) and males may contribute to late blight resistance of such progenies (Jellis, 1992; Stewart *et al.*, 1994).

Though most of the exotic accessions were either inferior or at par with Kufri Jyoti, a few accessions were better and had all the desired attributes *i.e.* resistance or moderate resistance to late blight, significantly higher yield than Kufri Jyoti and at par or better than Kufri Jyoti for tuber number and average tuber weight. These were CP1673, CP1690, CP2110, CP2378, CP2415, CP3098, CP3127, and CP3250. Except CP2415, which had pink tubers all these accessions had white tubers with acceptable tuber shape, eye depth, and uniformity. These accessions, however, need to be tested in large scale multilocation trials to pick up a few best for commercial cultivation.

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Use of Isozyme Polymorphism for Gender Evaluation in Kiwifruit (*Actinidia deliciosa* var. *deliciosa*)

Poonam Shirkot*, DR Sharma and CK Shirkot+**

Department of Biotechnology, +Department of Basic Sciences, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan-173230, Himachal Pradesh

This study demonstrates the usefulness and applicability of isozyme variation as sex markers for identification of staminate and pistillate cultivars of the dioecious kiwifruit (*Actinidia deliciosa* var. *deliciosa*). Seven cultivars of kiwifruit were surveyed for isozyme polymorphism by polyacrylamide gel electrophoresis using 11 enzyme systems. But only two enzyme systems peroxidase and catalase showed polymorphism leading to gender differentiation. The availability of enzyme markers for gender identification may be useful in kiwifruit breeding programmes and orchard plantations.

Key Words: Dioecious, Isozyme, Kiwifruit (*Actinidia deliciosa* var. *deliciosa*), Sex Markers

Kiwifruit (*Actinidia deliciosa* var. *deliciosa*), is a berry fruit crop indigenous to Northern and Central China and was commercially domesticated in New Zealand. (Ferguson and Eiseman, 1983). It is a perennial vine belonging to family Dilliniaceae and its dioecious nature provides a challenge for genetic and breeding research. McNeilage (1997) reported that *A. deliciosa* is hexaploid with 2n = 6x = 174. The pistillate plants are XXXXXX whereas staminates are XXXXY. Due to their small size, the X and Y chromosomes are not morphologically distinguishable and this single Y system allows the retention of dioecism.

Staminate and pistillate plants of some members of the genus *Actinidia* differ in vegetative characters (Li, 1952), but such differences are large and inconsistent. It would be an advantage to be able to determine the sex of seedlings without having to wait for 4 or 5 years before they flower. It may be possible to use biochemical tests in the form of isozyme for sex determination (Hirsch *et al.*, 1997; Deng *et al.*, 1982; Messina *et al.*, 1991).

Protein and isozyme patterns offer a quick and reliable system for identification of cultivars and are being used as fingerprints of genetic changes (Weeden and Lamb, 1985). Many authors have reported use of isozymes for verification of fruit cultivars at the nursery level. (Torres and Bergh, 1980; Hirai *et al.*, 1986; Weeden *et al.*, 1988; Sui *et al.*, 1995; Sanchez *et al.*, 1998). But there have been few attempts to identify kiwifruit cultivars as well as to study the sex determination by means of enzyme analysis (Messina *et al.*, 1991; Hirsch *et al.*, 1997).

** *Corresponding author*

Materials and Methods

The seven cultivars of *Actinidia deliciosa* var. *deliciosa* available in India and their source, used in the present study, are shown in Table 1. The material was obtained from the experimental orchard at Department of Pomology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan. Two staminate and five pistillate cultivars were used for the study.

Table 1. Name of cultivars, source and year of introduction in India

Name of Cultivar	EC No.	Year	Source
Allison (Male)	EC-24673	1963	USA
Allison (Female)	EC-24672	1967	USA
Bruno (Female)	EC-64090	1969	New Zealand
Hayward (Female)	EC-64093	1969	New Zealand
Abbott (Female)	EC-64094	1969	New Zealand
Tomuri (Male)	EC-64092	1969	New Zealand
Monty (Female)	EC-137263	1978	New Zealand

Source: Pandey and Joshi (1997)

Fresh swollen buds were excised from the seven cultivars of kiwifruit in the month of March-April and used immediately for extraction of soluble proteins for isozyme analysis as reported by Messina *et al.* (1991). Preliminary studies had eliminated young leaves as possible sample source due to poor resolution of isozymes and inconsistent staining. Only the stable, repeatable bands in each cultivar were scored. Enzyme extraction each methods used were as described earlier (Messina *et al.*, 1991). The samples were used immediately or stored at –4 °C for electrophoresis later on. For most enzymes, storage up to only 72 h was found to give

good resolution. Phosphoglucomutase (PGM) and phosphoglucoisomerase (PGI), however, were always analyzed in freshly prepared samples.

The 11 enzyme systems used in this study were resolved on four different gel buffer systems. The first buffer system was lithium borate. Tris citrate, a modification of Ashton and Braden's system (1961) comprising 0.04 M lithium hydroxide and 0.19 M boric acid, pH : 8.3 as an electrode buffer. The gel buffer consisted of 0.05 M Tris, 7 mM citric acid (pH : 8.3). This system was used for aspartate amino transferase (AAT) and phosphoglucomutase (PGM). Alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), catalase (CAT), peoxidase (PER), Esterase (EST) and phosphoglucoisomerase (PGI) were resolved using the continuous Tris borate buffer pH : 8.2 as modified by Poulik (1957). The electrode buffer contained 0.3 M boric acid and 0.06 M sodium hydroxide pH : 8.2 and gel buffer had 0.08 M Tris and 7 mM citric acid. Whereas, acid phosphatase (ACP) and alkaline phosphatase (ALP) and phosphorylase (PR) were resolved by a third system-Tris citrate buffer pH : 7.0 as modified by Meizel and Markert (1967) having 0.05 mM Tris and 0.016 M Citric acid as an electrode buffer and 0.017 M Tris and 0.005 M Citric acid in gel buffer.

Staining recepies for ADH, AAT, ALP and ACP were used as described by Shaw and Prasad (1970). Staining of PGI, PGM and MDH was done according to Soltis *et al.*. 1983 while the methods of Conkle *et al.*, (1981) were employed for EST and CAT. Staining procedures of Graham *et al.* (1964) were employed for PER and PR.

Polyàcrylamide gel electrophoresis was carried out to separate various isozymes due to its high resolving power, transparency and chemical inertness. Anionic system (Ornstein, 1964; Davis, 1964) was used for different isozyme systems.

Differences in enzyme banding patterns (phenotypes) were recorded as differences in relative mobility, the migration of each band relative to the protein front.

For statistical analysis, bands across all enzyme systems and entries were coded as present (1) or absent (0). Genetic similarities were calculated based on the method of Sneath and Sokal (1973) and relationship between entries were quantified using the complementary similarity indices. A dendrogram was constructed based on the genetic distance matrix data by applying the unweighted pair group method with arithmetic averages (UPGMA) using the numerical taxonomy and multivariate analysis system computer program (NTSYS-PC version 1.8).

Results and Discussion

Variability in banding patterns for differentiation of gender was observed only in two of the 11 enzyme systems used. The enzymes ADH, ALP, ACP, PGM and PR, showed identical banding patterns in all the cultivars and six enzyme systems *i.e.* MDH, PER, CAT, AAT, PGM and EST showed polymorphism. Sex-related polymorphism was depicted only by PER and CAT. On the basis of their banding patterns, of PER and CAT, the cultivars were divided into two classes of staminate and pistillate cultivars.

In peroxidase system four zones of activity appeared (Fig. 1). The fastest migrating one was monomorphic and showed a single band at RM 0.9 from the origin followed by another monomorphic band at RM 0.7. The male and female phenotypes could be distinguished through the presence or absence of a band at RM 0.60. It was found only in five female cultivars and absent in the male cultivars. Another monomorphic band was found in the anodal region at position RM 0.04.

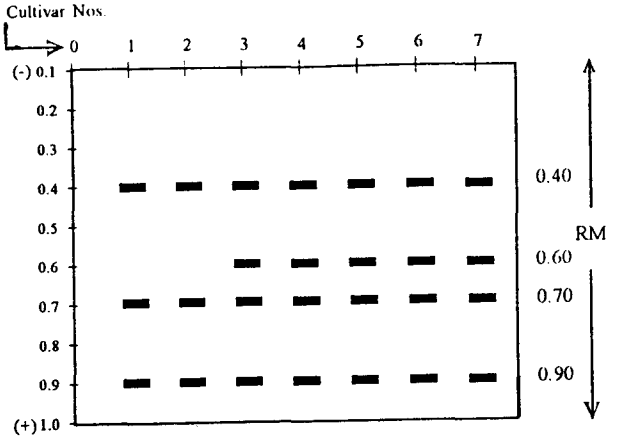

Fig. 1. Isozymic pattern of peroxidase (PER) enzyme (EC 1.11.1.7) in seven cultivars of Actinidia deliciosa var. deliciosa plant

Cultivars: 1. Tomuri male 2. Allison male 3. Hayward female 4. Bruno female 5. Monty female 6. Allison female 7. Abbot female

In the catalase isozyme system, four bands were distributed between positions RM 0.15 to 0.85 (Fig. 2). The perusal of the data revealed two types of banding patterns with bands at different positions. The first banding pattern was observed only in two male

cultivars, whereas, second banding pattern was specific to the five female cultivars. First band at position RM 0.85 was present in both male cultivars and absent in all the five female cultivars, whereas, second band at RM 0.75 was present only in the female cultivars. Third and fourth bands at positions RM 0.60 and 0.15 were monomorphic and overlapped in all the seven cultivars.

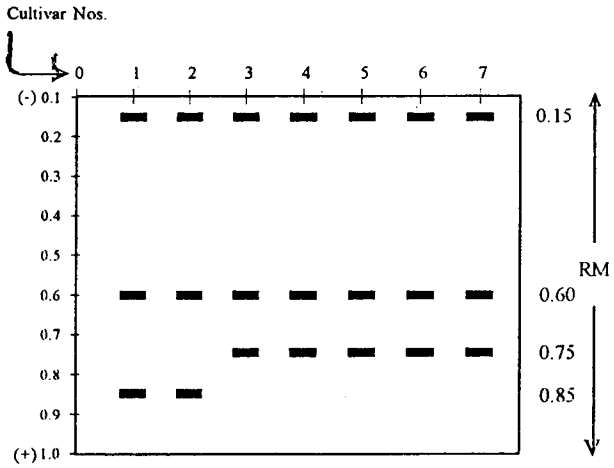

Fig. 2. Isozymic pattern of Catalase (CAT) enzyme (EC 1.11.1.6) in seven cultivars of Actinidia deliciosa *var.* deliciosa *plant*

Genetic similarity coefficients derived from pair-wise comparison among the pistillate and staminate cultivars are summarized in Fig. 3. The index value ranged from 0.73 to 0.88 with the mean of 0.81. The dendrogram showed two clusters of male and female cultivars. The cuitivar Allison (male) was found to be closer in distance to Tomuri than to any other cultivar. This indicates a high level of polymorphism, which is expected since the genus *Actinidia* is dioecious.

To our knowledge, not much genetic data based on biochemical markers are yet available on kiwifruit. However, Hirsch *et al.* (1997) have developed a peroxidase test for sex screening in young kiwifruit seedling. In their opinion, peroxidase may be useful to determine the sex of kiwifruit because this enzyme system is involved in the hormonal sex control in dioecious plants. Thus, present data are useful because the feasibility of such an analysis in this species is demonstrated. The availability of this material is particularly important for our study because in a dioecious species like *A. deliciosa* var. *deliciosa* individuals are expected to be highly heterozygous and this introduces an element of uncertainty in the cultivars. The present study shows distinction between male and female cultivars with respect to

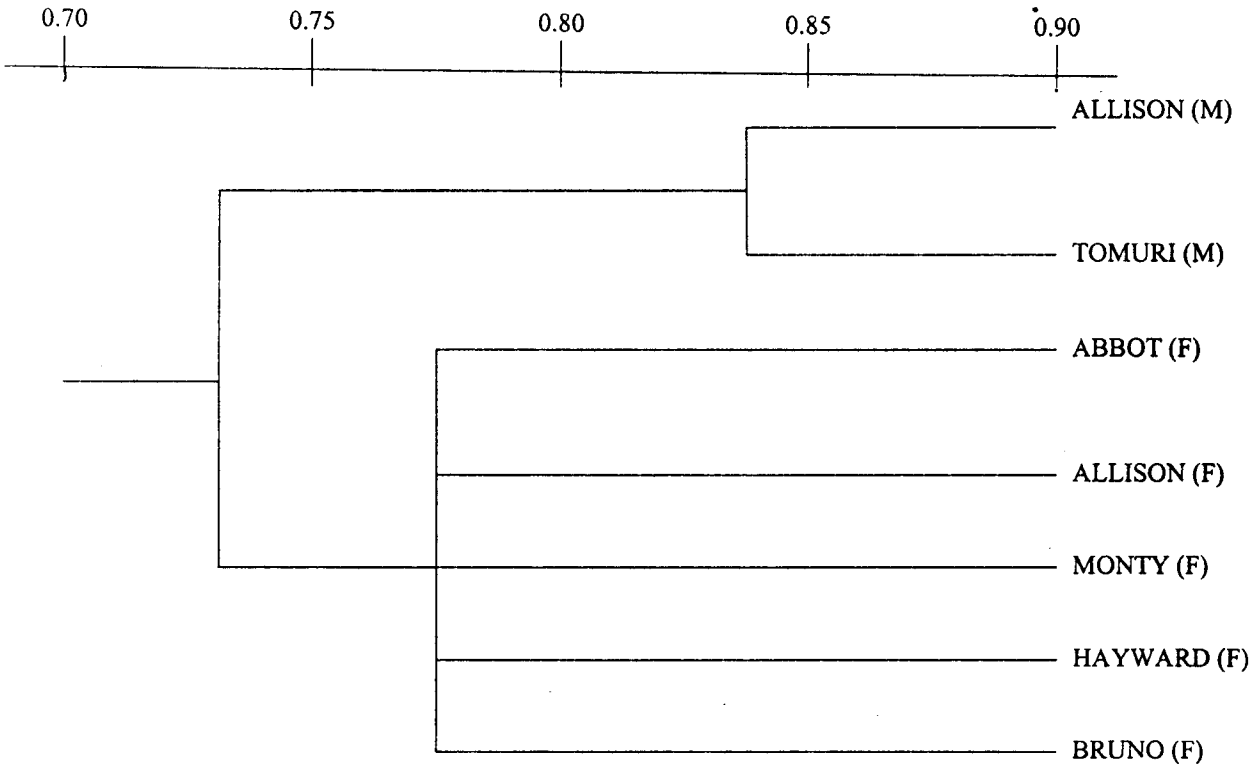

Fig. 3. Genetic relatedness among seven cultivars of Actinidia deliciosa var. deliciosa based on Peroxidase (PER) and Catalase (CAT) isozyme systems

peroxidase and catalase isozyme patterns. Catalases have so far not been used for sex determination of male and female seedlings. One band of peroxidase with RM 0.60 was observed only in five female cultivars (Fig. 1) suggested its female-associated nature. Also, one band of catalase with RM 0.85 consistently appeared only in male genotype (Fig. 2) suggesting thereby the male-associated nature of this marker.

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New Plant Type for High Yield Potential in Wheat-designed

SS Singh, JB Sharma, Nanak Chand, DN Sharma, JB Singh and Anita Baranwal
Division of Genetics, Indian Agricultural Research Institute, New Delhi-110012

To achieve a quantum jump in wheat productivity in India, there is a need to design a new plant type, combining negatively correlated yield components in a single genotype of very high yield potential. In this endeavour, new plant type combining two negatively correlated traits (high tillers with high grain weight and high grain weight with high grain number/ear) have been successfully combined resulting in significantly high yields. The second phase advance generation materials are in pipeline, which have optimum combination of all three yield components and also carry alien genes *(Lr24/Sr24)* for leaf and stem rusts resistance, would have further increased in yield potential.

Key Words: Plant Type, Rust Resistance, Wheat, Yield Components

Wheat production in India has increased due to development of varieties possessing high productivity for various agro-ecological zones of the country. The national average of wheat yield had increased from about one t/ha in early 1960s to nearly 27 t/ha in late 1990s. It is projected that by the year 2020, India will need 109 m t of wheat for internal consumption. To achieve this target, average yield must be increased from 2.7 t/ha to 4.0 t/ha. This increase in productivity is difficult to achieve through conventional wheat improvement approach of inter-varietal crosses. The other approach of tapping the variability existing in wild relatives of wheat, is also not yet perfected.

Since the last two decades, CIMMYT breeders are working on designing wheat architecture, named 'buitre type' with thicker stems, fewer tillers, larger heads and a higher number of grains without a commensurate decline in grains weight (Rajaram and Ginkel, 1996). Presently these positive traits are counter-balanced by an unknown physiological imbalance or disorder that results in a low number of tillers, largely sterile heads, mostly shrivelled grains and high susceptibility to leaf and stripe rusts (Pingali and Rajaram, 1999).

To further increase the yield potential, the uncommon approaches for exploiting the variability existing in local and diverse germplasm are to be used. Indian Agricultural Research Institute, New Delhi, initiated strategic research in 1994-95 by way of exploiting some local types characterized with very long spikes but shrivelled grains. These local types have shy tillering and are highly susceptible to rusts. The objective of utilizing these types was to develop wheat genotypes combining high grain weight with increased number of grains, optimum tillering and resistance to rusts. With the adoption of this strategy, several lines with new plant type were designed, resulting in very high yield and resistance to leaf and stem rusts.

Materials and Methods

The materials involved as parental lines, in the development of high yielding genotypes were local germplasm (SFW) and two released varieties (Vaishali and Vidisha) deriving leaf and stem rust resistance genes (*Lr24/Sr24*) from *Agropyron elongatum*.

The local germplasm was characterized by very long ear-head with unfilled middle spikelets, long and shrivelled grains, few tillers/plant and high susceptibility to rusts. This germpalsm line was included as one of the parents in crossing programme with a view to fill all the spikelets with bolder grains and combine other desirable traits from the second parent.

The other parents Vaishali and Vidisha, which were crossed with the local type, are released cultivars for timely sown irrigated conditions and late sown irrigated conditions in North Eastern Plain Zone and Central Zone, respectively. These parents were chosen with the objective to combine genes for resistance to stem and leaf rust and also to look for optimum combination of tillering habit, grain weight and grain number in single genotype. Along with this material four varieties namely PBW 343, HD 2687, HD 2329 and UP 2338 were included as check in the present investigation.

The new efficient plant type combining desirable yield components along with resistance to stem and leaf rusts were developed through modified pedigree method of selection from F_2 to F_5 filial generations. Two crosses of SFW (local type) with Vaishali and Vidisha were attempted and the F_1's were bulked. A very large F_2

population (approx. 2500 plants) of these two crosses was planted. The spreader rows were planted all around and in between, at regular intervals. The artificial epiphytotic of leaf rust was created by inoculating the spreader rows with the urediospores of most virulent race 77-5 with the help of hypodermal syringe. The selection in F_2 generation was exercised for plants combining maximum tillering, long and well filled ear heads and resistance to leaf rust. Selected F_2 plants were individually harvested and screened for well filled, bold and lustrous grains. F_3 families were raised from F_2 plants in 2.5 m x 2 rows plots. In selected F_3 families exhibiting resistance to rust and high tillering, long and well filled ear heads were picked up and threshed individually. In F_4 and F_5 generation ear row progenies were planted in epiphytotic conditions of leaf rust and long, well-filled ear heads were selected from the selected progeny rows having desired plant type and grain selection was exercised. The finally selected ear-heads from a progeny row were bulked and evaluated in Yield Trial comprising 6 rows of 5 m length with 3 replications evaluated in randomized block design during 1999-2000. One square meter plot from the middle of the plot of each entry in all replications were cut from the ground level when it was fully mature. The data on biological weight, grains yield, number of tillers were recorded from this one square meter plot area. Number of grains/ear-head were calculated from randomly selected 50 ear-heads from the harvested plot.

Data collected on various traits were analyzed for analysis of variance (ANOVA) and correlation among these traits. Correlation coefficient were calculated according to Miller *et al.*, (1958).

Results and Discussion

All the traits (grain yield/sq. m., number of tillers/ sq. m, 1000-grain weight and number of grains/ ear-head) except biological yield were highly significant which indicate very high variability in the material under study (Table 1).

Out of the nine newly developed lines, four lines yielded higher than all checks including the most popular variety PBW 343 in northern and western parts of the country (Table 2). Line DL 1266-2 has significantly out-yielded the best check variety PBW 343 and also both parental lines (SFW and Vaishali). This genotype is the ideal genotype with respect to optimum combination of tillers/plant (335), high grain weight (45.6 g) and number of grains/ear (46). This genotype also occupied first rank on the basis of mean yield/plot. According to new strategy of breeding genotypes combining optimum tillers/plant and higher two yield contributing traits (grain weight and number of grains), DL-1266-2 is the most suitable example.

Another genotype DL 1266-1 from the same cross also yielded higher than all the four checks and parental lines. However, it ranked 8th on the basis of grain yield/ plot. This genotype has the best combination of 1000 grain weight (51.5 g) and grains/year (50) but with low tillering habit. This type of genotype becomes the base material for further improvement in tillering habit combining with little low values of 1000 grain weight and grains/ear. DL 1266-6 is the third genotype of the same parentage, which is similar to DL 1266-2 in optimum combination of the entire yield contributing traits.

The fourth genotype DL 1280-1 from a cross between HD 2339 and Vaishali is also superior to all the check varieties. However, this genotype has a different combination of yield contributing traits. It has highest number of tillers/plant (611) and also very high 1000 grain weight (46.1 g) but less number of grains/ear (24). This genotype ranked second on the basis of grain yield/plot.

DL 1267-3 (SFW × Vidisha) yielded at par with the best check (PBW 343) has optimum combination of all the three yield contributing traits.

The local germplasm line (SFW) has low tillering (304) and low 1000-grain weight (33.3 g) and moderate

Table 1. Analysis of variance for yield and yield component

Source	D.F.	Mean sum of square				
		Biological yield	Grain yield	No. of tillers	1000-grain weight (g)	Grains/ear-head
Replication	2	12964.58	26787.62	4730.27	45.10	2.428
Treatments	15	35643.88	14111.24**	38830.13**	130.33*	198.51**
Error	30	28886.81	5053.33	2823.45	3.99	19.62

** Significant at 1%

Table 2. Mean values of yield and yield components

Varieties	Biological Yield/m²	Grain yield/m²	No. of tillers/m²	1000-grains wt.(g)	Grains/ ear	Plot yield (kg)
DL 1266-2	2166.00	707.83 (1)	335.33	45.60	46.44	4.320
DL 1266-1	1883.33	695.97 (2)	274.00	51.47	49.84	3.829
DL 1280-1	2113.33	667.77 (3)	611.00	46.13	23.79	4.134
DL 1266-6	1953.33	649.87 (4)	340.67	40.27	47.75	3.603
Vidisha (P)	2006.67	643.03 (5)	541.33	36.00	33.58	3.969
PBW 343 (C)	1956.67	625.47 (6)	514.67	33.47	36.40	4.125
DL 1267-3	1803.33	625.23 (7)	375.67	39.73	41.94	3.765
DL 1267-2	1956.67	625.17 (8)	376.67	39.73	41.97	3.778
HD 2329 (C)	1820.00	620.93 (9)	482.00	34.93	37.42	3.861
DL 1270-4	1950.00	610.80 (10)	487.33	38.00	33.19	3.830
DL 1266-3	1940.00	609.20 (11)	279.00	44.93	48.70	3.995
Vaishali (P)	1916.67	604.20 (12)	532.00	37.20	30.80	3.777
DL 1266-5	1806.67	601.73 (13)	222.00	48.67	56.03	3.781
HD 2687 (C)	2036.67	584.73 (14)	467.00	29.60	41.58	3.497
UP 2338 (C)	1930.00	553.87 (15)	446.33	29.47	42.51	3.627
SFW (P)	1723.33	402.27 (16)	303.67	33.33	40.40	2.702
CV (%)	8.76	11.57	12.90	5.08	10.86	–
SE	42.49	17.77	13.29	0.49	1.11	–
CD at 5%	283.4	118.5	88.60	3.33	7.38	–
CD at 1%	381.6	159.30	4.48	9.94	–	

P and C indicate parents and checks, respectively
Values within parentheses indicate ranks

grains/ear (40) but the number of spikelets/spike were very high which were poorly filled. It has been observed that the grains were very long but shrivelled resulting in low grain weight. The two parents Vaishali and Vidisha have higher tillers/plant and low grain weight and grain number/ear.

With the adoption of new strategy to have optimum combination of yield components DL 1266-2, DL 1266-1, DL 1266-6 (with common parentage) have been developed with new plant type where in the physiological efficiency of partitioning of the dry matter to economic yield has increased. This increase in physiological efficiency is due to increased availability of photosynthate for proper filling of sink leading to very high grain weight and proper filling of all the grains in all the spikelets resulting in higher number of grain/ear. In fact in SFW, the numbers of spikelets/spike are very high but grain formation is low because very poorly filled grains are highly shrivelled and unaccountable. The grains/ear in line DL 1280-1, which do not have SFW as one of the parent, is very low (24), further suggesting the role of SFW in contributing high grain number in newly developed genotypes. It is generally observed by several workers (Gandhi *et al.*, 1964; Bhatt, 1973; Chaudhary *et al.*, 1977; Sinha and Sharma, 1979; Balyan and Singh, 1987; Pawar *et al.*, 1990) that two yield contributing traits, grain weight and grain number/spike are negatively correlated. It is also known that the increase in tiller number/plant leads to decrease in seed weight and number of seeds/ear as is also evident from Table 3. However, the newly constituted plant type in the form of DL 1266-1, DL 1266-2, and DL 1266-6, have shown increase both in grain weight and grain number/ear. The significant positive correlation between grain weight and grains/ear is evident from this study (Table 3). It was also successfully possible to combine high tiller number and high grain weight in a genotype DL 1280-1, which are otherwise negatively correlated components of yield.

Table 3. Correction coefficient among yield and yield components

Character	Biological yield	Grain yield	No. of tillers	1000 grain wt.	Grains/ear
Biological yield	–	0.727**	0.402**	0.120	-0.129
Grain yield		–	0.212	0479**	0.086
No. of tillers			–	0.418**	0.874**
100-grain wt.				–	0.327*

* Significant at 5%; ** significant at 1%

The concerted efforts are on in the directions of amalgamating two positive combination of yield components present in DL 1266 group and DL 1280-1, along with optimising selection criteria leading to maximizing the productivity. This advance material in pipeline has passed through preliminary yield trials and are under testing in replicated multi-location trials.

The *Agropyron elongatum* derived leaf rust resistance gene *Lr24* is effective till today in Indian sub-continent. This leaf rust resistance gene is linked with stem rust resistance gene *Sr24* which is effective to an array of virulent and prevalent races in the country. All the newly developed genotypes in the study carry this combination of leaf and stem rust resistance genes, introgressed through two released wheats Vaishali and Vidisha, the carrier of these genes *i.e. Lr24/Sr24*. All these genotypes were found highly resistant to leaf and stem rusts when tested as seeding in the glasshouse and as adult plants at hot spots.

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Strategies for Developing Core Collections of Safflower (*Carthamus tinctorius* L.) Germplasm – Part I. Sampling from Diversity Groups of Quantitative Morphological Descriptors

KK Suresh and R Balakrishnan
Department of Statistics, Bharathiar University, Coimbatore-641046, Tamil Nadu

Evaluation data collected from a germplasm collection of 3,250 safflower (*Carthamus tinctorius* L.) accessions was used to divide the whole collection into six major clusters based on multivariate cluster analysis. The clusters were further grouped into 30 diversity groups based on the geographical origin of the accessions and the plant types. Estimates of the phenotypic diversity of the core samples were obtained through simple random sampling and 5 stratified random sampling strategies. They were compared for varying sampling fractions ranging from 5% to 25% of the whole collection. In the core samples obtained through simple random sampling or stratified random sampling by frequency proportion method or diversity proportion method, the pooled diversity index based on 28 descriptors was close to the diversity of the whole collection. However, when the accessions from different diversity groups were allocated with equal frequency or in proportion to the logarithm of the number of accessions in each group or in proportion to the square root-proportion of the number of accessions in each group, the resultant core samples had higher levels of diversity than the whole collection. About 10-15% of the whole collection was found to be adequate as the core sample size.

Key Words: Core Sample, Information Measure, Kullback Divergence Statistics (KDS), Safflower, Shannon Diversity Index (SDI), Stratified Sampling

The potential use of plant genetic resources contained in large germplasm collections could be greatly enhanced by constituting sub-samples called core collections. The broad guideline for constituting a core sample as suggested by Frankel (1984) is that, it should include the maximum of genetic variation contained in the whole collection with minimum duplication. Obviously, the quality of the core sample is dependent upon good passport and evaluation data on the accessions that constitute the whole collection. Constituting a core sample of reasonable size and sufficient diversity by making use of available data on the accessions has been one of the most important issues of germplasm management. The basic issues that are of primary concern are (i) Core sample size, and (ii) Sampling strategies that could help in selecting accessions that reproduce the variation for several characters in the whole collection to the maximum possible extent. Brown (1989 a, b) addressed the issue of optimum core sample size and suggested that about 10 per cent of the whole collection would allow preservation of about 80% of alleles in a large collection. Methods for obtaining core samples using different sampling strategies had been suggested by several workers. They were mainly concerned with grouping of accessions into homogenous groups or clusters and selecting sub samples from each group to obtain a pooled core sample. The grouping approaches described were hierarchical (Hintum TJL Van, 1995; Peeters and Martenelli, 1989) or non-hierarchical cluster analysis methods using quantitative or a mixture of both quantitative and qualitative descriptors (Spagnoletti Zeuli and Qualset, 1993; Mahajan *et al.*, 1996; Harch *et al.*, 1996; Bisht *et al.*, 1998). Grouping of the accessions based on their geographical origin had also been suggested by several authors. The most common method of sampling is simple random sampling from each group to obtain a core sample of desired size. Several strategies had also been suggested for deciding appropriate sampling fraction from each group or strata. These methods included proportional allocation, log frequency allocation, square-root frequency proportion allocation etc. (Brown 1989b; Spagnoletti Zeuli and Qualset, 1993; Mahajan *et al.*, 1999; Balakrishnan and Suresh, 2000). As an alternative to simple random sampling, Noirot *et al.*, (1996) suggested that the accessions could be ranked on the basis of their relative contribution to the overall variance and a desired proportion of top ranked accessions could be selected from each group to constitute the core sample. This approach was found to be very useful not only in obtaining core samples of reasonable size, but also the diversity in terms of several qualitative descriptors were almost equal to that of the whole collection (Mahajan *et al.*, 1996; Balakrishnan *and Suresh* 2000).

In the present investigation, the diversity in a large germplasm collection of 3,250 safflower accessions has been studied for a set of 18 qualitative and 10 quantitative descriptors. These accessions were grouped into 6 major clusters based on the morphological and agronomic characters. These major clusters were further divided into 30 diversity groups based on the geographical origin and plant type of the accessions. Simple random sampling and stratified random sampling with 5 methods of group allocation in the core samples were used to compare the diversity of the core sample with that of the whole collection. An information measure was computed to study how far the resultant core sample deviated from the joint density distribution of the whole collection with respect to the set of descriptors. A measure of divergence of the core sample from the reserve collection or the non-core group was also evaluated under the various sampling strategies.

Materials and Methods

The data set on safflower (*C. tinctorius* L.) pertains to a collection of 3,250 accessions that were grown and evaluated at the Germplasm Management Unit of the All India Coordinated Research Project on Oilseeds at Solapur, Maharashtra, India (latitude: 17°14' N and longitude: 75°56' E). The majority of these accessions were from India and the remaining accessions from various other countries that were obtained from the world genebank through the courtesy of late Dr. PF Knowles and which were later assembled from different coordinating centres at the gemplasm management unit at Solapur. The material was raised during 1987-1990 in single rows of 6m length spaced at 45 cm between rows and 35 cm within rows in augmented block design under protective irrigation. Ghorpade *et al.*, (1991) had published a comprehensive catalogue of the passport and evaluation data of these accessions. For the present investigation, ten quantitative and 18 qualitative traits from this data set were considered for the purpose of constituting the core set from this germplasm collection.

The list of descriptors used for the present investigation is provided in Table 1. The number of accessions from different geographical regions varied widely, the majority of the accessions coming from India (2444) followed by United States of America (330). There were far lesser number of accessions from other countries and they are presented in Table 2.

Table 1. List of descriptors used for the study

1. Shape of lower stem leaf : Lanceolate narrow, lanceolate broad
2. Margin of lower stem leaf: Serrate, deeply serrate, entire, deeply lobed
3. Primary head shape before flowering: Conical, flattened, oval
4. Texture of upper leaves: Fleshy, normal, leathery
5. Shape of upper stem leaf: Lanceolate narrow, lanceolate broad, oblong
6. Margin of upper stem leaf: Entire, serrate, slightly serrate, few serrate, deeply lobed
7. Spines on upper stem leaf: Non-spiny, few spines, intermediate, many spines
8. Attitude of OIB* to head: Closed, open
9. OIB* cross section shape: Flat, grooved
10. Location of spines on OIB*: None, tip only, tip & few basal, tip & few apical, tip & all along margin
11. Number of spines on OIB*: None, intermediate, many
12. Length of spines on OIB*: None, short, intermediate, long
13. Bracts enclosing head: Complete, incomplete
14. Growth habit: Bushy, cone shaped, appressed, erect
15. Branch location on main stem: Primarily basal, upper 1/3, upper 2/3, base to apex
16. Pollen production: Sparse, intermediate, abundant
17. Pappus on the acheme: Present, absent, negligible
18. Hull thickness: Thin/ intermediate, thick
19. Days to 50% elongation: 20-23, 23-26, etc.,..... 38-41, > 41
20. Days to primary branch initiation: 30-35, 35-40, etc., ... 55-60, > 60
21. Days to first flowering: <= 55, 55-60, etc., ...95-100, >100
22. Days to 50% flowering: <= 65, 65-70, etc., ... 105-110, > 110
23. Days to physiological maturity: 70-80, 80-90, etc., ...140-150, > 150
24. Plant spread (cm): 10-20, 20-30, etc.,..... 80-90, > 90
25. Number of primary branches: <= 3, 3-6, etc.,..... 24-24, > 27
26. Number of capitula per plant: 0-10, 10-20, etc., ... 90-100, >100
27. Mean inter-node length (cm) : 1, 2, etc., ...,10
28. Main Capitula diameter (cm): 0.5-1.0, 1.0-1.5, etc., ...3.0-3.5, >3.5

* OIB: Outer Invocular Bracts

Cluster analysis

A random sample of 325 accessions (10%) from the whole collection was initially subjected to cluster analysis using Ward's method. For the purpose of cluster analysis, the attribute data for the qualitative descriptors were converted into numerical scores based on the method suggested by Balakrishnan and Sanghvi (1968). These numerical scores depend on the dispersion matrix of

Table 2. Region-wise breakup of accessions under major clusters of safflower germplasm collection

Geo. Region	Major clusters						
	1	2	3	4	5	6	Total
IND	795	1298	88	33	225	5	2444
USA	3	125	41	66	84	11	330
IRN	0	9	10	7	22	14	62
PAK	3	20	3	2	37	2	67
SUN	1	4	4	0	21	2	32
EUR	0	10	2	2	17	1	32
ESP	0	4	11	0	14	1	30
AFR	0	23	1	0	17	3	44
EGY	0	8	11	2	7	0	28
TUR	0	18	9	7	25	10	69
M.E.	0	9	6	4	7	7	33
AUS	0	12	1	2	3	0	18
Others	0	20	18	1	20	2	61
Total	802	1560	205	126	499	58	3250

IND: India and Bangladesh
USA: USA, Canada and Mexico
IRN: Iran and Iraq
PAK: Pakistan and Afghanistan
SUN: Russia, Germany and Poland
EUR: Switzerland, France, United Kingdom, Hungary and Italy
ESP: Spain and Portugal
AFR: Algeria, Ethiopia, Kenya, Libya, Morocco and Sudan
EGY: Egypt
TUR: Turkey
M.E: Israel, Syria, Lebanon and Jordan
AUS: Australia

the individual descriptors. The dispersion matrix was computed for each descriptor by pooling the frequency densities of the attribute values over 13 different geographical regions. The method is more objective in that it preserves the distance among various groups and avoids arbitrary scaling of the qualitative attributes into numerical values. The values for the 10 quantitative traits were standardized before subjecting them to cluster analysis. An initial set of 6 clusters was identified from this sample of 325 accessions. The remaining accessions were allocated to these clusters based on two methods, *viz.*, (i) The Euclidean distance method and (ii) the classification scheme of Boulton and Wallace (1970). For the Euclidean method, the cluster centres were computed and the Quick Cluster procedure of SPSS software package was used to allocate the remaining accessions into the initial clusters. For the Boulton-Wallace method, the joint density distributions of the descriptors under the 6 initial clusters were computed and any accession that had the highest probability of being nearest to any cluster was assigned to that cluster. An information measure (Wallace and Boulton, 1968) was also computed to assess the better of the two classification schemes. Based on this, the clustering scheme of Boulton and Wallace was considered for the data set of 3250 safflower accessions.

The number of accessions in each of the six main clusters and their distribution over different geographical regions is presented in Table 2. For further dividing these major clusters into diversity groups, geographical origin of these accessions (whether they belong to India, USA or others) and the plant growth type (bushy, cone shaped, erect or appressed) were taken into consideration. The diversity groups are presented in Table 3.

Diversity index

For computing the diversity index using the Shannon formula, the numerical descriptors were converted into appropriate class intervals and each class interval was treated as a descriptor state. For the i^{th} descriptor if P_{ij} is the proportion of its j^{th} attribute state in the population, then the population Shannon Diversity Index (SDI) for that descriptor can be computed using the formula:

$$SDI_i = -\sum_{j=1}^{s} P_{ij} * Log_e(P_{ij})$$

A pooled diversity index SDI across all the 28 descriptors was then computed by adding the SDI values for the individual descriptors. Similarly in case of computing the pooled diversity index for a core sample of a given size, the same formula was used by replacing the population proportion P_{ij} with the sample proportion P_{ij} for a given descriptor state.

Estimation of mean and variance of the pooled SDI through sampling

For obtaining core samples, 5 different sizes were considered. They were approximately fixed at 5, 10, 15, 20 and 25% of the whole collection and were respectively 150, 330, 480, 660 and 810. For drawing samples from each of the 30 diversity groups, simple random sampling and stratified random samples were considered. For drawing the samples through stratified random sampling, five methods of allocation were considered. They were:

1. Frequency proportion (FP method): In this method, the number of accessions drawn from any group was in proportion to the group size.
2. Square root -proportion (SQP method): In this method if P_i was the proportion of accessions from

i^{th} group in the whole collection, then q_i, the proportion allocated to that group in the core sample was computed as $q_i = \sqrt{p_i} / [-\sum_{i=1}^{s}(\sqrt{p_i})]$, *s*, being the number of groups.

3. Log frequency (LF method): In this method if F_i is the number of accessions in the i^{th} group in the whole collection then, the proportion q_i allocated to that group in the core sample was computed as: $q_i = \text{Log } F_i / [\sum_{i=1}^{s}(\text{Log } F_i)]$, *s* being the number of groups).
4. Equal frequency (EF method): In this method equal number of accessions were selected from each group for a given size of the core sample.
5. Diversity Proportion (DP method): In this case the number of accessions selected from each group depended on the proportion of the pooled diversity of that group i.e. the proportion of $(N_i * SDI_{st(I),})$ where N_i is the number of accessions in the i^{th} group and $SDI_{st(I)}$ is the pooled SDI of that group. The diversity index for each group is also provided in Table 3.

The pooled SDI being a complex parameter, to estimate its expected value and sampling variance, for the method of simple random sampling, 100 independent random samples of a given size were drawn without replacement from the given data set. In case of stratified random sampling the same procedure was followed and it was ensured that the number of accessions from each group were fixed as per the method of allocation. The sample pooled SDI was computed in each case and also the mean and variance of the SDI was computed over the 100 samples. Also the mean SDI for the individual descriptors for the core sample as well as for the unselected accessions (non-core group) was computed. Mahajan *et al.*, (1999) suggested an estimator with appropriate weights for the individual groups for estimating the pooled SDI when the stratified random sampling was used. The weights may have to be fixed such that this index approached the value of the population SDI. But in the present investigation our main interest was only in the diversity of the core sample *per se.* Hence, the resultant core sample was treated as one homogeneous mixture of accessions from different diversity groups and the properties of the core sample were studied in terms of the mean and variance of the pooled SDI. Also, the closeness of the joint density distribution of the descriptors in the core sample to that of the whole collection was evaluated by means of an information measure. This is explained below.

Table 3. Diversity groups based on geographical origin and plant types

Main cluster	Group ID	Description entries	No. of SDI	Group
1	01	From India - Bushy	253	14.76
	02	.....- Cone shaped	455	15.10
	03	.....- Erect/appressed	94	14.86
2	04	From India-Bushy	648	15.72
	05	.....- Cone shaped	378	19.94
	06	.....- Erect/appressed	272	19.21
	07	From USA – Bushy	69	18.16
	08	.....- Cone shaped	35	20.03
	09	..... – Erect/appressed	21	21.44
	10	Other regions-Bushy	36	21.07
	11	.....- Cone shaped	55	21.30
	12	.....- Erect/appressed	46	20.35
3	13	From India – Bushy/cone shaped	50	23.39
	14	.....- Erect/appressed	38	23.88
	15	From USA – Bushy/cone shaped	24	25.46
	16	.....- Erect/appressed	17	24.55
	17	Other regions – Bushy/cone shaped	22	23.62
	18	.....- Erect/appressed	54	26.34
4	19	Mostly Erect type...India	33	22.70
	20	.....USA	66	22.56
	21	.....Other regions	27	25.15
5	22	From India – Bushy	37	20.47
	23	.....- Cone shaped	99	21.63
	24	.....- Erect/appressed	89	23.92
	25	From USA – Bushy	12	19.28
	26	.....- Cone shaped	26	23.18
	27	.....- Erect/appressed	46	25.06
	28	Other regions – Bushy/cone shaped	33	24.50
	29	.....- Erect/appressed	157	25.80
6	30	Mostly erect/appressed type	58	25.40
		Pooled SDI for whole collection		26.14

Following Boulton and Wallace (1970), for each multi-state or qualitative descriptor '*d*' the probability of occurrence of descriptor state '*m*' of the attribute in the whole collection is estimated by

$$p[m,d] = \{n[m,d] + 1\}/\{n[d] + M[d]\} \quad (1)$$

where n[m,d] denotes the number of accessions having attribute state *m* of the descriptor *d*; n[d], the number of accessions having any known value of the attribute *d*; and M[d], the number of descriptor states of descriptor *d*. In general, if data on all attributes *d* are available

on all the accessions, n[d] would be equal to the number of accessions in the whole collection. Based on information theory concepts, the length of the information code that can optimally indicate the possession of descriptor state *m* of attribute *d* is equal to

$$c[m,d] = -\log_e p[m,d] = -\log_e \{n[m,d] + 1\}/\{n[d] + M[d]\} \quad (2)$$

The estimate (1) is slightly biased to prevent the divergence of (2) when n[m,d] = 0, and has the useful effect of allowing an accession to be taken into consideration without much error even though it has a descriptor state *m* not possessed by any existing member of that group. For each accession *s* in the core sample, a message length F[s] that is required to optimally encode all the *d* attributes of *s* using the joint density distribution of the whole collection, is then computed using the formula:

$$F[s] = \sum_{des} c[x[d,s],d], \quad (3)$$

where $\sum_{des}$ means summing over all discrete or qualitative attributes; and *x*[d,s] indicates any descriptor state *m* of attribute *d* possessed by the accession. The message length c[x,d] is obtained from (2) and hence the total message length that is attributed to each of the accessions in a core sample of a given size can be computed. The average message length can therefore be computed over the 100 repeated random samples that are drawn by the sampling procedure. The mean message length would depend on the size of the core sample. Any sampling scheme that gives the least mean message length for a given sample size would have the joint density distribution of the descriptors closest to that of the whole collection.

The deviation of the frequency patterns in the core sample from those of the whole collection was also tested. Since, the core samples formed a part of the whole collection, a direct comparison was not possible. Hence, the frequency patterns of the individual descriptors in the core sample as well as for the non-core group were compared by means of a chi-square test (Rao, 1973). Such a test implies the statistical significance of the differences between the diversity of the core sample and the whole collection. Such deviations in the diversity measure of the individual descriptors can be pooled over and can be evaluated by computing the statistic:

$KDS = \sum_{des} \{\sum_{j=1}^{s} (p_{1j} - p_{2j}) \log_e (p_{1j} / p_{2j})\}$, called Kullback Divergence Statistic (Goldstein and Dillon, 1978), where p_{1j} and p_{2j} represent the relative frequency of the j^{th} descriptor state of any descriptor in the core sample and the non-core group respectively and *s* being the number of descriptor states and $\sum_{des}$ indicating summation over all the descriptors. In order to obtain valid estimates of KDS in cases where the core sample or the non-core group may have zero frequency for a descriptor state, a slightly biased estimate of p_{ij} were used as shown in equation (1) which did not result in any appreciable error. The mean KDS values were computed for the core samples obtained by various schemes and their values compared. The magnitude of the mean KDS would indicate the divergence of the core sample from the non-core group and hence from the whole collection.

Results and Discussion

The number of accessions from different geographical regions that are grouped into six major clusters is presented in Table 2. Cluster 2 was the largest cluster with nearly 50% of the accessions, followed by Cluster 1. Cluster 6 was the smallest with 58 accessions. Out of the 802 accessions in Cluster 1, 795 were from India, which has the largest number of accessions in the whole collection. In Cluster 2, apart from a large number of accessions from India, there were a large proportion of accessions from USA also. Similarly, in Clusters 3, 4 and 5, the accessions from these two regions had a larger proportion of accessions. However, Cluster 6 did not show any predominant region and this cluster was taken as a single diversity group. In Table 3, the grouping of accessions into 30 diversity groups is shown based on their geographical origin and the plant growth habit.

The pooled diversity SDI for the whole collection was equal to 26.14 and the group-wise diversity index as shown in Table 3 indicated wide variation among the diversity groups. Even though groups-1,2 and 3 had fairly large number of accessions, the diversity in these groups was quite small as compared to that of the whole collection. This may be due to the fact these groups have mostly accessions from India, where a large number of duplicates were suspected. Diversity groups obtained from Cluster 2 had only moderate diversity and even in this case a majority of the accessions were from India. Cluster 3, which had smaller number of accessions from India, USA and other countries had a diversity index approaching that of the whole collection. Cluster 4 indicated moderate diversity from the groups containing accessions from India and USA, but showed a diversity approaching the population diversity for the group

consisting of accessions from other countries. Again in Cluster 5, groups consisting of accessions from USA and other countries had a diversity that was close to that of the whole collection. Cluster 6, consisting mostly of erect plant types had a diversity index close to that of the whole collection. In general, the groups consisting of accessions from India, the main source of origin of safflower, had relatively smaller diversity compared to the groups that consist of accessions from other countries. Balakrishnan and Suresh (2000) indicated that in a core collection of about 570 safflower accessions from this data set, nearly 45% of the accessions from India were probably duplicates in view of a very high similarity index among them. This could be due to the fact that the Indian accessions had been pooled from different coordinating centres within a narrow geographical area, mostly from the states of Maharashtra, Karnataka and Andhra Pradesh which are the predominant regions of safflower cultivation.

Using a pooled estimator for the SDI by combining the stratum diversity indices with the weights proportional to the group size, resulted in the estimate of the pooled diversity equal to 18.94, much smaller than the actual population diversity index of 26.14. Since the main interest of the present investigation was to assess the diversity of the core samples obtained through various stratification schemes, each of the core samples was treated as a homogeneous mixture of accessions from different diversity groups and the pooled diversity in the core samples were directly computed. The mean and variance of the pooled diversity index estimated using 100 random samples (of appropriate sizes) are presented in Table 4. Core samples constituted by simple random sampling, FP method and DP method had almost the same level of pooled diversity as that of the whole collection. In these methods, the variance of the pooled SDI reduced with increased sample size and about 10-15% of the sample could be considered optimum for these methods beyond which reduction in variance was not appreciable. In the case of SQP, LF and EF methods, the diversity indices of the core samples progressively increased with increasing sample size. Core samples obtained through the EF method had maximum diversity even at 15% of the sample size. All these three methods yielded higher pooled diversity for the core samples than the whole collection. This result would not have been obvious if we had differentiated the individual diversity groups in the resultant core sample and used an estimator with weights. In contrast to this Mahajan *et al.*, (1999) indicated in their results that the weighted-index was about 75-80% of the population SDI. Table 4 also indicated that by using SQP, LF or EF methods, the core sample size could be kept at 10% of the whole collection, beyond which there was not much appreciable reduction in the variance of the estimated pooled SDI.

The information measure presented as the average message length in Table 4 also indicated that simple random sampling, FP method and PD method (in that

Table 4. Diversity measures for the core samples drawn from the whole collection by simple random sampling and stratified random sampling

Sample size	Mean SDI*	Variance (SDI)	Mean Message Length	Mean KDS**
1. Simple random sampling				
150	25.72	0.3876	3917	0.855
330	26.00	0.2163	8637	0.402
480	26.00	0.1230	12535	0.290
660	26.04	0.0900	17239	0.221
810	26.10	0.0671	21195	0.193
2. Stratified - Frequency proportion method				
150	25.97	0.1336	3945	0.770
330	26.07	0.0509	8651	0.347
480	26.13	0.0376	12587	0.251
660	26.00	0.0289	17201	0.190
810	26.00	0.0153	21098	0.163
3. Stratified - Square root proportion method				
150	28.28	0.1068	4362	1.327
330	28.50	0.0581	9606	1.560
480	28.58	0.0228	13992	1.624
660	28.62	0.0211	19253	1.798
810	28.65	0.0142	23637	1.971
4. Stratified - Log frequency method				
150	29.22	0.1147	4589	3.179
330	29.57	0.0335	10175	3.186
480	29.60	0.0209	14783	3.361
660	29.60	0.0130	20296	3.755
810	29.57	0.0101	24860	4.168
5. Stratified - Diversity proportional method				
150	26.98	0.1218	4115	1.115
330	27.08	0.0420	9017	0.646
480	27.08	0.0393	13096	0.572
660	27.11	0.0237	18001	0.535
810	27.13	0.0192	22101	0.560
6. Stratified - Equal frequency method				
150	29.85	0.0885	4770	4.402
330	30.03	0.0260	10494	4.417
480	30.01	0.0223	15204	4.664

* - Pooled Shannon Diversity Index based on 28 descriptors
** - Kullback Divergence Statistic measuring the divergence of the core sample from the non-core group

order) had the least average message lengths for any given core sample size. This evidently indicated that the core samples obtained by these methods had their joint density distribution for the descriptors as close to that of the whole collection. Because of this the pooled diversity of the core samples obtained by these methods were nearly equal to that of the whole sample. The core samples obtained through EF, LF and SQP methods (in that order) had larger message lengths for any given core sample size. This indicated that the joint density distribution of the core samples obtained by these methods were farther away from that of the whole collection, resulting in a higher diversity index for the core samples.

The mean divergence of the core samples from the reserve collection (non-core group) similarly showed interesting trends. In case of simple random sampling, FP and PD methods, the KDS values were low indicating that the frequency patterns in the core samples were similar to those of the reserve collection and the divergence *decreased* with increasing sample size. However, in the case of SQP, LF and EF methods, not only the core samples were more divergent from the non-core group, but also, this divergence increased with increasing sample size. The EF method had the largest amount of divergence for any given sample size.

A more detailed analysis of the deviation of the core sample from the whole collection in terms of SDI for the individual descriptors is presented in Table 5. The results have been presented for the core sample size of 10% of the whole collection and the SDI values are presented in a standardized form so that its value has a range of 0-1. The results clearly indicated that the diversity levels of the core samples obtained by simple random sampling, stratified random sampling using frequency proportion and diversity proportion methods were at par with those of the whole collection for all the descriptors. Core samples obtained using the square root proportion, log frequency and equal frequency methods of stratified random sampling had significantly higher levels of diversity than the whole collection for many descriptors. Interestingly, core samples obtained through equal frequency method had maximum diversity for several descriptors.

The general conclusion from the present investigation was that sampling of the accessions through stratification based on the diversity groups was more efficient than simple random sampling. This was due to the fact that sampling variances of the pooled SDI estimated through the five stratification schemes were much smaller than that of the simple random sampling method. The core samples obtained by frequency proportion method of stratified random sampling had nearly the same level of diversity as that of simple random sampling but the sampling variance of the estimate of pooled SDI was much less than that of simple random sampling. The diversity proportion method which was meant to give due importance to diversity levels of the individual groups as well as their size resulted in core samples with marginal increase in the estimated pooled SDI and lesser sampling variance. The other three methods of stratified random sampling not only yielded core samples with higher levels of diversity but also had reduced sampling variances for the estimate of pooled SDI. The square root proportion and log frequency methods of allocation were mainly aimed at reducing undue weights given to larger groups that might contain higher levels of genetic redundancy. As the larger groups in the present study had relatively lesser diversity than the smaller groups, these two methods resulted in core samples with higher diversity than the whole collection. Of all the six methods considered in the present study, the equal frequency method of allocation resulted in core samples with maximum diversity. This was due to the fact that the smaller groups had larger levels of diversity. Other strategies like the diversity dependent strategy without regard to group size (Yonezawa *et al.*, 1995) and the GL strategy based on the proportion of the product of logarithm of group size and group diversity (Mahajan *et al.*, 1999) yielded similar results to those of the DP strategy and the LF strategy respectively. Hence these results are not reported here.

The present study indicated that by appropriate stratification of the accessions and group allocation in the core sampling methodology, it was possible to obtain a core sample that might contain higher level of diversity than the whole collection. This leads us to examine whether it would be possible to predict by how much the diversity level of the core sample is likely to exceed that of the whole collection. Obviously, it could be difficult to predict the expected improvement in diversity of the core sample based on any one of the above sampling strategies. The results could vary depending upon the diversity levels of individual descriptors in different diversity groups; the group sizes and the sampling

Table 5. Mean diversity indices for descriptors in the core samples obtained using different sampling strategies at 10% core sample size

Descriptor name	Whole collection	Simple random	FP method	DP method	SQP method	LF method	EF method
Shape of lower stem leaf	0.544	0.541	0.548	0.550	0.568	0.592	0.609
Margin of lower stem leaf	0.322	0.325	0.319	0.337	0.378	0.391	* 0.421
Primary head shape	0.376	0.376	0.368	0.435	**0.522	**0.593	**0.627
Texture of upper leaves	0.624	0.627	0.629	0.653	*0.694	**0.694	**0.732
Shape of upper stem leaf	0.395	0.393	0.402	0.453	* 0.480	**0.515	**0.535
Margin of upper stem leaf	0.535	0.532	0.541	0.577	**0.627	**0.668	**0.686
No. of spines on upper stem leaf	0.520	0.518	0.521	0.569	**0.630	**0.682	**0.715
Attitude of OIB to head	0.731	0.731	0.734	0.804	**0.890	**0.951	**0.971
OIB cross section shape	0.600	0.600	0.600	0.668	**0.775	**0.840	**0.884
Location of spines on OIB	0.248	0.242	0.241	0.297	**0.379	**0.443	**0.475
No. of spines on OIB	0.534	0.533	0.531	0.594	**0.698	**0.767	**0.810
Length of spines on OIB	0.607	0.602	0.612	0.663	**0.744	**0.800	**0.832
Bracts enclosing head	0.319	0.322	0.320	0.372	**0.480	**0.545	**0.597
Growth habit	0.893	0.892	0.896	0.914	**0.932	**0.944	**0.949
Branch location on main stem	0.809	0.806	0.805	0.835	*0.848	**0.858	**0.869
Pollen production	0.822	0.818	0.828	0.848	0.870	*0.896	**0.914
Pappus on the acheme	0.246	0.234	0.248	0.248	0.268	0.276	0.290
Hull thickness	0.638	0.636	0.646	0.651	0.695	0.716	*0.740
Days to 50% elongation	0.682	0.683	0.681	0.694	*0.712	**0.715	**0.706
Days to primary branch initiation	0.704	0.705	0.703	0.717	*0.729	**0.733	**0.727
Days to 1st flowering	0.809	0.811	0.811	0.825	**0.843	**0.849	**0.844
Days to 50% flowering	0.807	0.810	0.812	0.828	**0.850	**0.858	**0.854
Days to physiol. maturity	0.650	0.653	0.653	0.661	**0.676	**0.664	**0.648
Plant spread	0.704	0.703	0.701	0.718	0.721	0.734	0.740
No. of primary branches	0.723	0.724	0.721	0.723	0.732	0.733	0.743
No. of capitula/plant	0.684	0.684	0.684	0.697	0.714	0.726	0.746
Internode length	0.558	0.563	0.565	0.571	0.578	0.591	0.596
Main capitula diameter	0.557	0.554	0.559	0.574	0.598	*0.623	**0.634
No. of accessions	3250	330	330	330	330	330	330

*, ** - Mean proportions of accessions in the core sample with different attributes of the descriptor significantly different from those of the whole collection at 5% and 1% probability levels, respectively

method. The basic objective was to reproduce almost the same level of genetic diversity in the whole collection through a smaller collection called the core sample. But practical considerations suggest that a user of the germplasm collection might require a core sample with desired or pre-determined levels of diversity for one or more descriptors simultaneously, preferably with much higher levels of diversity than the whole collection itself. As an example, an attribute (say, spines on leaves) may be present in 10% of the accessions and absent in 90% of the accessions in the whole collection. This indicates a standardized SDI of 0.47 (range 0-1) for this descriptor. Can the user of the genetic resource obtain a core sample with say, the attribute being present in 70% of the accessions and absent in 30% of the accessions in the core sample (with a standardized SDI of 0.88)? This implies not only much higher level of the diversity in the core sample, but also an entirely different distribution pattern of the descriptor states in the core sample. If the diversity level of only one descriptor is pre-determined, then it is quite simple to draw such a sample. However, when the diversity levels of several descriptors that are both qualitative and quantitative are simultaneously pre-assigned for the core sample, none of the sampling strategies considered above serve this objective as these methods involve only random selection. Hence it requires an entirely different approach to be adopted for obtaining such a core sample. In Part II of this investigation a new method is proposed for obtaining a core sample with pre-determined distribution patterns for one or more descriptors simultaneously.

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Appendix I

Converting a qualitative attribute to numerical score (Refer Balakrishnan and Sanghvi, 1968 for more details)

Let p_{ijk} be the estimated proportions of the k^{th} descriptor state of the j^{th} character S_j in the i^{th} group or stratum ($i = 1,2.....q$; $k = 1,2.....s_j + 1$). The dispersion matrix of the estimates p_{ijk} is given by $\mathbf{A}_{ij}$, where

$A_{ijk} = p_{ijk} (1- p_{ijk})/n_{ij}$, $k = l$

$= -p_{ijk} * p_{ijl} /n_{ij}$, $k \neq l$,

$k, l = 1,2,.....s_j +1$ and

n_{ij} is the sample size for the j^{th} character from i^{th} group. Since $\sum p_{ijk} = 1$ ($k = 1$ to $s_j +1$); the rows and columns of $\mathbf{A}_{ij}$ add up to 0.

The proportions may be taken as the means of variables X_{jk} which take only the values 0 and 1, subject to the condition that one and only one of the X_{jk}'s can be unity on any individual.

The common dispersion matrix of the variable X_{jk} over the q groups is estimated by $\mathbf{C}_j$, where

$$C_{jkl} = \sum_{i=1}^{q} n_{jk}^2 A_{ijkl} / \sum_{i=1}^{q} n_{ij}$$

Having computed the common dispersion matrix, the procedure of obtaining the transformed variables in terms of the original variables is given by Rao (1952, chapter 9), The only requirement is that we have to ignore or delete any row and the corresponding column of $\mathbf{C}_j$ before the process. The procedure is illustrated with the following example.The pooled dispersion matrix of the descriptor *'Number of spines on upper stem leaf'* with the descriptor states: No spines, Few, Intermediate and Many (pooled over the 13 geographical regions shown in Table 2) was:

0.0376 -0.0102 -0.0263 -0.0010

-0.0102 0.1197 -0.1056 -0.0039

-0.0263 -0.1056 0.1518 -0.0205

-0.0010 -0.0039 -0.0205 0.0254

Eliminating the first row and column corresponding to 'No spines' and using the procedure of Rao (1952), the transformed variables for the descriptor states are:

No: Y(1) = 0 (because we have not considered this row and column).

Few: Y(2) = 2.8904 X(2), where X(2) = 1 when this attribute is present

Int: Y(3) = 3.6431 X(2) + 4.1296 X(3), where only X(3) =1 for this attribute; X(2) = 0

Many: Y(4) = 3.1549 X(2) + 3.2794 X(3) + 8.0325 X(4), where only X(4) = 1 for this attribute and X(2) = X(3) = 0

As one and only one attribute can take a value of 1 when it is present, in the above equations the numerical scores for the 4 qualitative attributes are: 0, 2.89, 4.13 and 8.03 respectively. Thus this scoring system is more objective than the arbitrary scaling of 0,1,2 and 3 for the above attributes as it takes into consideration the dispersion matrix of the attributes' frequency in the population.

We may arbitrarily choose any row and the corresponding column to be ignored. But by convention, we may omit the row/column corresponding to the lowest ranked attribute in the case of an ordered-multi state descriptor (as in the present example). In the case of binary attributes the scores are given as 1 and 0 as usual.

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Strategies for Developing Core Collections of Safflower (*Carthamus tinctorius* L.) Germplasm – Part II. Using an Information Measure for Obtaining a Core Sample with Pre-determined Diversity Levels for Several Descriptors Simultaneously

R Balakrishnan and KK Suresh
Department of Statistics, Bharathiar University, Coimbatore-641046, Tamil Nadu

A new technique for establishing a core sample from a large germplasm collection based on an information measure is proposed. The procedure is viewed as a classification scheme having two groups, *viz.*, the core sample group and the non-core group with pre-assigned properties in terms of group sizes and the density distributions of several descriptors. That is, for a known or fixed core sample size, the proportion p[m,d,t] of each state *m* for each multi-state qualitative descriptor *d* is pre-assigned within each group *t*. Similarly, for each continuous or quantitative descriptor *d*, the mean μ[d,t] and standard deviation σ[d,t] are pre-assigned. The probability of finding an accession with a given set of attributes or values in the vicinity of either of the two groups is estimated from the density of the pre-assigned composite model for each group. Then, based on an information measure a group is identified that has the highest density in the neighbourhood of any accession in the measurement space and each accession in the collection is assigned to that group *i.e.* the core sample or the non-core group. The information measure that is used is the length of the message that can optimally convey the description of the measurements, which equals the negative value of the logarithm of the probability of obtaining the given set of measurements in the neighbourhood of any one of the two groups. An accession that has the least length with regard to any group is assigned that group. The method is illustrated with a data set of safflower germplasm described by Balakrishnan and Suresh (2000) and Part I of this article. A scheme by which the accessions are stratified as per their geographical origin is also considered. The diversity indices of the core samples constituted by using various options in the proposed method are compared with those of the whole collection as well as those obtained through simple or stratified random sampling.

Key Words: ***Carthamus tinctorius*** **L., Core Collection, Information Measure, Shannon Diversity Index (SDI)**

One of the major issues about which a germplasm manager must be concerned is the need to improve the accessibility of the collections of a genetic resource to the users. In practice, plant breeders who are the main users of the germplasm collections are interested in having fairly small number of genotypes which possess or likely to possess the characters needed in their breeding programmes. For this purpose simple random sampling of the accessions in the main collection may not be adequate as the users wish to identify unique genetic resources. Rather, the sample should be biased to assure the inclusion of accessions from poorly represented geographic regions/diversity groups or should provide a reasonable amount of diversity in morphological traits and variation in metric traits. In part I of this article, we considered an approach that used passport, characterization and other data of a safflower germplasm data set containing 3,250 accessions (Ghorpade *et al.*, 1991) to first identify diversity groups of accessions and then select random samples from each group. It was found that core samples with higher levels of diversity than the whole collection could be obtained by some of the stratified random sampling methods. Nevertheless it was pointed out that the properties of the resultant core sample might vary depending upon the diversity levels of the individual descriptors in different groups, the group sizes and the method of sampling. Therefore, a different approach is required if the user of the genetic resource wants to have a core collection of desired size with pre-determined frequency patterns for two or more descriptors, preferably with higher levels of diversity than the whole collection. The present investigation suggests such a procedure by which the user can fix the properties of the core sample in terms of diversity measures of several qualitative and quantitative descriptors simultaneously.

Materials and Methods

The safflower germplasm data set as detailed in Part I of this article was used for developing the procedure for obtaining a core sample with pre-determined levels of diversity for 15 descriptors as listed in Table 1. In order to pre-assign the diversity of the core sample in

terms of individual qualitative and quantitative descriptors the following computations were made.

Table 1. List of descriptors used for the study

1. Margin of lower stem leaves: Serrate, deeply serrate and deeply lobed
2. Texture of upper leaves: Fleshy, normal, leathery
3. Spines on upper stem leaf: Non-spiny, few spines, intermediate, many spines
4. Location of spines on OIB*: None, tip only, tip & few basal, tip & few apical, tip & all along margin
5. Number of spines on OIB*: None, intermediate, many
6. Length of spines on OIB*: None, short, intermediate, long
7. Bracts enclosing head: Complete, incomplete
8. Growth habit: Bushy, cone shaped, appressed, erect
9. Pollen production: Sparse, intermediate, abundant
10. Hull thickness: Thin/ intermediate, thick
11. Days to physiological maturity: 70-80, 80-90, etc., ...140-150, > 150
12. Number of primary branches: <= 3, 3-6, etc., ...24-24, > 27
13. Number of capitula per plant: 0-10, 10-20, etc., ... 90-100, >100
14. Mean inter-node length (cm) : 1, 2, etc., ...,10
15. Main Capitula diameter (cm): 0.5-1.0, 1.0-1.5, etc., ...3.0-3.5, >3.5

* OIB: Outer Invoclucre Bracts

(a) ***Qualitative Descriptors***

The relative frequencies of accessions in the whole collection with respect to the individual descriptors were computed. In order to have a higher level of diversity in the core sample, two types of frequency transformations were used, *viz.*, the square root-proportion and the log-frequency transformations. For the square root-proportion transformation, if p_i was the relative frequency of a descriptor state in the whole collection, the relative frequency q_i in the core sample for that descriptor state was computed as $q_i = \sqrt{p_i} / [\sum_{i=1}^{s} \sqrt{p_i}]$, where s is the number of descriptor states for a specific descriptor. For the log-frequency transformation, if F_i was the absolute frequency of a descriptor state in the whole collection, the relative frequency q_i in the core sample for that descriptor state was computed as $q_i = \log(F_i)/[\sum_{i=1}^{s} \log(F_i)]$. These two frequency transformations have a useful property that descriptor states with high relative frequency in the whole collection have reduced relative frequency in the core sample and those with low relative frequency in the whole collection have somewhat higher relative frequencies in the core sample. By these methods, the diversity level for a descriptor in the core sample as measured by Shannon Diversity Index (SDI) was improved substantially if the SDI was low in the whole collection for this descriptor. For a given pre-assigned core sample size, the relative frequencies of various attributes in the core sample and the non-core group could therefore be easily computed. It was ensured that in case the absolute frequency for a descriptor state in the core sample exceeded the corresponding frequency in the whole collection, the frequency for that descriptor state in the core sample was made equal to that of the whole collection. The excess number of accessions was added to the largest frequency class in the core group. This implied minor modifications in the relative frequencies for the descriptor states for both the groups. In Table 2 and Table 3, the computations have been illustrated for a sample of qualitative descriptors by fixing the core sample size as 420 using the square root-proportion and log frequency transformations respectively. It should be noted that once the q_i's are fixed (that are independent of the core sample size), then the corresponding relative frequencies in the non-core group will depend on the size of the core sample and the relative frequencies in the whole collection. The core sample size of 420 was fixed for this illustration based on the results of Balakrishnan and Suresh (2000) for the safflower data set. Similar computations for a core sample of 10% of the whole collection (325) were made but not illustrated here.

The Shannon Diversity Index (SDI) for each descriptor for the core sample and the non-core groups was computed by using the formula:

$$SDI = -\sum_{i=1}^{s} p_i \log_e (p_i),$$

where p_i were estimated as $(n_i + 1)/(n + s)$, n_i being the frequency of a particular descriptor state; n- the total number of observations and s- the number of descriptor states for a particular descriptor. The estimates of p_i are slightly biased to enable a descriptor state with zero frequency to be included in the computation of the SDI though it results in a negligible error. The SDI value was divided by its maximum possible value, i.e. by $\log_e(s)$, so that its value ranges from 0 to 1.

(b) ***Quantitative Descriptors***

The results of principal component method arrived at by Balakrishnan and Suresh (2000) for the safflower data were used for fixing the frequency patterns of the quantitative descriptors. Based on this study, a core sample of 420 accessions was recommended for the safflower germplasm after eliminating probable duplicates from the first stage core sample of nearly 570 accessions. This core sample had higher diversity for the quantitative

Table 2. Examples of fixing the descriptor state frequencies of the core sample as per the square-root proportion transformation

Descriptor & descriptor states	Freq. In whole collection*	Relative frequency in the whole collection	Relative frequency fixed for the core sample*	Freq. in non-core group**	Relative frequency in non-core group
Texture of upper leaves					
Fleshy	62	0.0191	0.0917	23	0.0081
Normal	2260	0.6954	0.5536	2028	0.7166
Leathery	928	0.2855	0.3547	779	0.2753
Standardized SDI		0.6245	0.8366		0.5776
Location of spines on OIB					
None	133	0.0409	0.1346	77	0.0272
Tip only	62	0.0191	0.0919	23	0.0081
Tip & few basal	88	0.0271	0.1095	42	0.0148
Tip & few apical	6	0.0018	0.0143	0	0.0000
Tip & all along the margin	2961	0.9111	0.6496	2688	0.9499
Standardized SDI		0.2517	0.6748		0.1589
Geographical origin					
India & Bangladesh	2444	0.7520	0.3570	2294	0.8106
USA, Canada & Mexico	330	0.1015	0.1312	275	0.0971
Iran & Iraq	62	0.0191	0.0569	38	0.0135
Pakistan & Afghanistan	67	0.0206	0.0591	42	0.0149
Russia, Germany & Poland	32	0.0098	0.0408	15	0.0052
Switzerland, France, U.K., Hungary, Italy	32	0.0098	0.0408	15	0.0052
Spain & Portugal	30	0.0092	0.0396	13	0.0047
Algeria, Ethiopia, Kenya, Libya, Morocco & Sudan	44	0.0135	0.0479	24	0.0084
Egypt	28	0.0086	0.0382	12	0.0042
Turkey	69	0.0212	0.0600	44	0.0155
Israel, Syria, Jordan & Lebanon	33	0.0102	0.0415	16	0.0055
Australia	18	0.0055	0.0306	5	0.0018
Other countries	61	0.0188	0.0564	37	0.0132
Standardized SDI		0.4161	0.8627		0.3271

* Size of the main collection = 3250; Size of core sample = 420
** Size of non-core group = 2830

descriptors than the whole collection and the same frequency pattern as found in the resultant core sample was used for fixing the diversity of the quantitative descriptors in the present study. A sample of frequency patterns for days to physiological maturity and number of primary branches/plant are presented in Table 3. For further analysis in this investigation, the quantitative descriptors were categorized as qualitative descriptors with appropriate class-intervals being treated as the descriptor states.

Two approaches were followed for constituting the core samples. In the first approach a combination of 10 qualitative and 5 quantitative descriptors with pre-assigned frequency distributions were considered (the detailed list as provided in Table 1) with a core sample of 420 accessions. In the second approach only the 10 qualitative descriptors were considered with pre-assigned frequency densities for the core sample and the core sample size was fixed at 10%, *i.e.* 325 accessions. Some of the morphological descriptors were selected based on their association with reaction of the germplasm material to *alternaria* leaf spot disease (Venkateswara Rao *et al.*, 1993) and safflower aphids (Balakrishnan *et al.*, 1994). Three of the descriptors *viz.*, margin of lower stem leaves, location of spines on OIB and bracts enclosing head were selected in view of low diversity for them in the whole collection. Growth habit and hull thickness had fairly high level of diversity in the whole collection. The quantitative traits were included based on their agronomic importance. Under both these approaches, two options were tried in fixing the frequency patterns of the qualitative descriptors, *viz.*, (a) the square

Table 3. Examples of fixing the descriptor state frequencies of the core sample as per the log-frequency transformation

Descriptor & descriptor states	Freq. in whole collection*	Relative frequency in the whole collection	Relative frequency fixed for the core sample*	Freq. in non-core group**	Relative frequency in non-core group
Texture of upper leaves					
Fleshy	62	0.0191	0.1476	0	0.0000
Normal	2260	0.6954	0.4867	2056	0.7264
Leathery	928	0.2855	0.3657	774	0.2736
Standardized SDI		0.6245	0.9123		0.5370
Location of spines on OIB					
None	133	0.0409	0.2101	45	0.0158
Tip only	62	0.0191	0.1476	0	0.0000
Tip & few basal	88	0.0271	0.1923	7	0.0026
Tip & few apical	6	0.0018	0.0143	0	0.0000
Tip & all along the margin	2961	0.9111	0.4357	2778	0.9816
Standardized SDI		0.2489	0.8437		0.0676
Geographical origin					
India & Bangladesh	2444	0.7520	0.1547	2379	0.8406
USA, Canada & Mexico	330	0.1015	0.1072	285	0.1007
Iran & Iraq	62	0.0191	0.0763	30	0.0106
Pakistan & Afghanistan	67	0.0206	0.0777	34	0.0121
Russia, Germany & Poland	32	0.0098	0.0640	5	0.0018
Switzerland, France, U.K., Hungary, Italy	32	0.0098	0.0640	5	0.0018
Spain & Portugal	30	0.0092	0.0629	4	0.0013
Algeria, Ethiopia, Kenya, Libya, Morocco & Sudan	44	0.0135	0.0699	15	0.0052
Egypt	28	0.0086	0.0616	2	0.0008
Turkey	69	0.0212	0.0782	36	0.0128
Israel, Syria, Jordan & Lebanon	33	0.0102	0.0646	6	0.0021
Australia	18	0.0055	0.0429	0	0.0000
Other countries	61	0.0188	0.0760	29	0.0103
Standardized SDI		0.4161	0.9811		0.2646

* Size of the main collection = 3250; Size of core sample = 420; ** Size of non-core group = 2830

root-proportion method and (b) the log-frequency method.

In order to group the accessions as a basis for stratified sampling, strata of geographical origin of the accessions were taken into consideration. For this, the passport data of the accessions was used. The stratification method was included in the two approaches described above by simply considering an additional variable, *viz.*, geographical origin by treating it as yet another qualitative descriptor with descriptor states being the codes for the source country of the accessions. Again, in order to allow for a better representation of accessions from geographical regions with a very small proportion of accessions in the whole collection, the square root-proportion method and the log-frequency methods were followed to pre-determine the frequency patterns of the geographical regions in the core sample. The pre-assigned frequencies for accessions in the core sample from different geographical regions are also presented in Tables 2 and 3.

In effect, eight schemes were considered for constituting the core samples that are referred to as Scheme-1 to Scheme-8 in further discussions. They are:

Schemes	Method of fixing frequency distribution of the core samples and the core sample size
Scheme-1	Frequency patterns of 10 qualitative descriptors as per square root-proportion method + pre-assigned frequency distribution of 5 quantitative descriptors; no stratification: pre-assigned core sample size = 420
Scheme-2	Same as Scheme-1 but with stratification of accessions as per their geographical origin with their allocation as per square root-proportion method
Scheme-3	Frequency patterns of 10 qualitative descriptors as per the square root-proportion method; no stratification: pre-assigned core sample size = 325 (10% of whole collection)
Scheme-4	Same as Scheme-3 but with stratification of accessions as per their geographical origin
Scheme-5	Frequency patterns of 10 qualitative descriptors as per log-frequency method + pre-assigned frequency distribution of 5 quantitative descriptors; no stratification : pre-assigned core sample size = 420
Scheme-6	Same as Scheme-5 but with stratification of accessions as per their geographical origin with their allocation as per log-frequency method
Scheme-7	Frequency patterns of only 10 qualitative descriptors fixed as per the log-frequency method; no stratification: pre-assigned core sample size = 325
Scheme-8	Same as Scheme-7 but with stratification of accessions as per their geographical origin with their allocation as per log-frequency method

Table 4. Examples of frequency profiles of some quantitative descriptors in the core sample and the non-core sample group

Descriptor	Core sample		Non-core group	
	Absolute frequency	Relative frequency	Absolute frequency	Relative frequency
No. primary branches/plant				
≤ 3	6	0.0143	12	0.0042
3 - 6	82	0.1952	545	0.1926
6 - 9	104	0.2476	996	0.3519
9 - 12	82	0.1952	626	0.2212
12 - 15	43	0.1024	375	0.1325
15 - 18	48	0.1143	222	0.0784
18 - 21	16	0.0381	44	0.0155
21 - 24	26	0.0619	7	0.0025
24 - 27	4	0.0095	1	0.0004
> 27	9	0.0215	2	0.0008
Total	420	1.0000	2830	1.0000
Standardized SDI		0.8546		0.6969
Days to physiological maturity				
70- 80	1	0.0024	0	0.0000
80- 90	3	0.0071	1	0.0004
90-100	5	0.0119	78	0.0276
100-110	11	0.0262	477	0.1685
110-120	61	0.1452	1325	0.4682
120-130	149	0.3548	717	0.2533
130-140	140	0.3333	219	0.0774
140-150	48	0.1143	12	0.0042
150-160	2	0.0048	1	0.0004
Total	420	1.0000	2830	1.0000
Standardized SDI		0.6934		0.6093

Having fixed the frequency patterns of the descriptors for the core sample group and the non-core group, the next step was to compute appropriate information measure. This information measure served as the criterion for the classification of individual accessions with a given set of attribute values into the core sample or the non-core group. The details of the theoretical framework and development of this information statistic has been explained by Wallace & Boulton (1968). An algorithm has been proposed by Boulton and Wallace (1970) that can easily be adapted to our present objective of allocating an accession to the core sample group or the non-core group. The computational aspects of this algorithm are explained below.

1. For each multi-state or qualitative descriptor '*d*' the probability of occurrence of descriptor state '*m*' of the attribute in group '*t*' (t =1 meaning the core sample group and t = 2 meaning the non-core group) is estimated by

$$p[m,d,t] = \{n[m,d,t] + 1\}/\{n[d,t] + M[d]\} \quad (1)$$

where n[m,d,t] denotes the number of accessions in group *t* having attribute state *m* of the descriptor *d*; n[d,t], the number of accessions in group *t* having any known value of the attribute *d*; and M[d], the number of descriptor states of descriptor *d*. In general, if data on all attributes *d* are available on all the accessions, n[d,t] would be equal to the number of accessions in group *t*. This is fixed beforehand by assigning the core sample size. The length of the information code that can optimally indicate the possession of descriptor state *m* of attribute *d* is equal to

$$c[m,d,t] = -\log_e p[m,d,t] = -\log_e \{n[m,d,t] + 1\}/\{n[d,t] + M[d]\} \quad (2)$$

The estimate (1) is slightly biased to prevent the divergence of (2) when n[m,d,t] = 0, and has the useful effect of allowing an accession to be assigned to a group without much error even though it has a descriptor state *m* not possessed by any existing member of that group.

2. For each continuous or quantitative descriptor *d*, the distribution is assumed to be normal within each group *t*. Its mean is estimated by

$$\mu[d,t] = (\Sigma_t\, x[d,k])/n[d,t] \quad (3)$$

where Σ_t indicates summation over the entries in group *t* and *x*[d,k] indicates the value of accession *k* on attribute *d*. The standard deviation is estimated by

$$\sigma[d,t] = \{(\Sigma_i(x[d,k])^2 - n[d,t]\ (\mu[d,t])^2)/ (n[d,t]\ -1)\}^{0.5} \quad (4)$$

A distribution normalizing constant g[d,t] is estimated by

$$g[d,t] = \log_e\ (\sigma[d,t]/(K^*\ \varepsilon[d])) \quad (5)$$

where $K = 1/\sqrt{2\Pi}$. It is assumed that a measurement $x[d,k]$ of the attribute d of an accession k is quoted to a least count of $\varepsilon[d]$, i.e. to an accuracy of $\pm\varepsilon[d]$ and that the probability of getting such a measurement from the distribution (μ,σ) is approximately

$$(K^*\ \varepsilon[d]/\sigma)^*\ \exp(-(x[d,k]\ -\ \mu)^2/2\sigma^2)$$

3. For each accession k, a message length F[k,t] that is required to optimally encode all the d attributes of k using the density distribution of group $t(t = 1 \text{ or } t = 2)$, is then computed using the formula:

$$F[k,t] = I[t] + \Sigma_{des}\ c[x[d,k],d,t] + \Sigma_{qty}\ (g[d,t] + (x[d,k]\ -\ m[d,t])^2\ /\ 2(s[d,t])^2) \quad (6)$$

where Σ_{des} means summing over all discrete or qualitative attributes; Σ_{qty} means summing over all continuous or quantitative attributes and $x[d,k]$ indicates any descriptor state m of attribute d possessed by the accession k in the case of a qualitative descriptor and it indicates the numerical value in the case of a quantitative descriptor. It is also assumed that the variables under consideration are independent of each other. The first parameter I[t] in (6) is an estimate of the message length for the group label to which an accession belongs. This depends on the abundance of group t in terms of the number of accessions in that group and is estimated by

$$I[t] = \log_e(S/n[t]),$$

where S is the total number of accessions in the whole collection (=3250 for the present case) and n[t] is the pre-assigned size of group t. For the present study, n[1] is the core sample size (= 420 or 325) and n[2] is the non-core group size (=2830 or 2925). In the present study the quantitative traits with appropriate class-intervals were treated like qualitative descriptors and hence equations (3) to (5) and the third component of (6) were not used.

For each accession k in the whole collection, depending upon the values possessed by it on various descriptors, the value of F[k,t] is computed for each of the two groups *viz.*, the core sample group and the non-core group. If $F(k,1) \leq F(k,2)$ then the accession is assigned to the core sample, else it is assigned to the non-core group. The computations are illustrated as follows. Let us assume that a core sample of 420 accessions is required with frequency densities for 2 of the descriptors as shown in Table 2a and no stratification is involved. In Table 4 the values of c[m,d,t] are shown for these two sample descriptors along with the descriptor state frequencies in the core sample and non-core group. By using the values in Table 4, it can be seen that any accession possessing descriptor state 'Fleshy leaves' for the descriptor 'texture of upper leaves' and 'Tip and few basal' for the descriptor 'location of spines on OIB' has the values F[k,1] = I(1) + 2.3838 + 2.2019 = 6.6319 and F[k,2] = I(2) + 4.7302 + 4.1886 = 9.0572. Here, $I(1) = \log_e(3250/420) = 2.0462$ and $I(2) = \log_e(3250/2830) = 0.1384$ are the message lengths that correspond to the group abundance for the two groups, *viz.*, the core sample and the non-core group. Hence the accession is allocated to group t = 1 i.e. the core sample, for F(k,t) has a minimum value for t =1.

Let us assume that the selection scheme involves stratification of the accessions as per their geographical origin and the expected region-wise allocation in the core sample is as shown in Table 2a. We now take the message lengths that correspond to the stratum or group to which an accession belongs and add them to the above values and the accession is assigned to the core sample or the other group depending upon the new computed values. For example, if an accession from USA region possesses the above two attribute values, then F(k,1) = 6.6319 + 2.0454 = 8.6773 and F(k,2) = 9.0572 + 2.3322 = 11.3894, where 2.0454 and 2.3322 are the message lengths for the two groups that correspond to USA region (Table 4). Hence it is assigned to the core sample group as F(k,1) is still less than F(k,2). However, when more number of descriptors belonging to both qualitative and quantitative types are taken into consideration, the assignment to either of the two group might change depending upon the values of c[m,d,t] for each group. The above procedure is repeated for all the accessions in the whole collection and each accession is allocated to either of the two groups.

It should be pointed out that even though we specify the initial core sample size, the final core sample size might vary to some extent depending upon the actual allocation of the accessions to either group based on the above procedure. If the resultant core sample size is lesser than the pre-assigned size, this core sample could be treated as the final product as it is still likely to be close to the pre-assigned frequency patterns or diversity levels. However, if the resultant core sample

Table 5. Computation of message length c[m,d,t] for encoding the attribute values under the core sample and the non-core group – an illustration

Descriptor & descriptor states	Frequency in core sample*	Frequency in non-core group*	c[m,d,t] for core sample	c[m,d,t] for non-core group
Texture of upper leaves				
Fleshy	39	23	2.3838	4.7302
Normal	232	2028	0.5921	0.3343
Leathery	149	779	1.0367	1.2893
Location of spines on OIB				
None	56	77	1.9916	3.6060
Tip only	39	23	2.3632	4.7717
Tip & few basal	46	42	2.2019	4.1886
Tip & few apical	6	0	4.2603	7.2566
Tip & all along margin	273	2688	0.4390	0.0529
Geographical origin				
India & Bangladesh	150	2294	1.0535	0.2141
USA, Canada & Mexico	55	275	2.0454	2.3322
Iran & Iraq	24	38	2.8519	4.2891
Pakistan & Afghanistan	25	42	2.8126	4.1914
Russia, Germany & Poland	17	15	3.1804	5.1800
Switzerland, France, U.K., Hungary & Italy	17	15	3.1804	5.1800
Spain & Portugal	17	13	3.1804	5.3136
Algeria, Ethiopia, Kenya, Libya, Morocco & Sudan	20	24	3.0262	4.7337
Egypt	16	12	3.2375	5.3877
Turkey	25	44	2.8126	4.1460
Israel, Syria, Jordan & Lebanon	17	16	3.1804	5.1194
Australia	13	5	3.4317	6.1609
Other countries	24	37	2.8519	4.3150

Size of the whole collection = 3250; Size of core sample = 420; Size of non-core group = 2830

* Frequency patterns as illustrated in Table 2.

size far exceeds the expected size (as is the case in the present study), the accessions allocated to the core sample could be ranked in ascending order based on the F(k,1) values and the required number of accessions as per their ranking could be taken out of this core sample and the rest allocated to the non-core group or the reserve collection. Since F(k,1) is the message length that corresponds to an accession being in the vicinity of the core sample with pre-assigned joint density of the descriptors, the required number of accessions that have shorter message length F(k,1) could be selected to the core collection and the remaining ones with larger values of F(k,1) could be relocated to the reserve collection. In this method, if fairly large number of accessions are left out of the first stage core sample, it is quite likely that the frequency patterns in the reduced core sample might yield somewhat lesser diversity than the pre-assigned one for one or more descriptors. An alternate but a cumbersome method is to split the first stage core sample into 2 groups, one with the pre-assigned size and the other with the balance number of accessions and repeat the whole procedure again with pre-assigned higher levels of diversity to obtain a second stage core sample. This procedure after a few iterations would result in a core sample that is quite close to the pre-assigned diversity index for several descriptors simultaneously. However, this procedure might be attempted if suitable computer program is available to perform the computations.

The diversity of various descriptors as measured by Shannon Diversity Index (SDI) was computed for the core samples obtained by various schemes. The SDI values were divided by $\log_e(n)$ to make it range from 0 to 1, where n stands for the number of descriptor states. The deviation of the frequency patterns in the core samples obtained from different schemes from those of the whole collection was also tested. Since, the core samples form a part of the whole collection, a direct comparison was not possible. Hence, the frequency

patterns of the individual descriptors in the core sample were compared against those in the non-core group by means of a chi-square test (Rao, 1973). Such a test implies the statistical significance of the differences between the diversity of the core sample and the whole collection.

In order to compare the results of the new method with the simple random sampling method, accessions were drawn at random from the whole collection without replacement to obtain 100 independent core samples of sizes 325 and 420, respectively. The mean SDI was computed for each descriptor from these 100 samples. Also the same procedure was repeated for stratified random sampling with strata of 13 geographical regions with the proportions of allocation as listed in Table 2 (square root-proportion method) and Table 3 (log-frequency method) for the geographical regions. The SDI values for the descriptors in the core samples obtained through simple random sampling or stratified random sampling were compared with those obtained through the new procedure using a t-test (Poole, 1974).

Results and Discussion

Core samples were obtained based on the 8 schemes (as listed under the earlier section). Scheme 1 resulted in a first stage core sample of 510 accessions against the pre-assigned size of 420 accessions. Similarly scheme 2, 5 and 6 resulted in first stage core samples of size 542, 514 and 556 respectively against the pre-assigned size of 420. Hence, 420 accessions were retained in the core sample for each of these schemes based on the ascending order values of F(k,1). Schemes 3,4,7 and 8 resulted in first stage core samples of size 359, 408, 407, 408 respectively against the pre-assigned size of 325 accessions. Out of these first stage core samples only the required number of 325 accessions were retained as per the ordering of F(k,1) values.

The standardized SDI values for various descriptors under consideration are presented in Table 6 for the core samples obtained through schemes 1, 2, 5 and 6 (sample size = 420). For comparison, the SDI values for the whole collection and the mean diversity for core samples obtained by simple random sampling (SRS) and stratified random sampling with strata of geographical origins are also included in this table.

A perusal of Table 6 indicates that without stratification of the accessions, the simple random sampling (SRS) method yielded a core sample with mean SDI values of the descriptors that are almost equal to those of the whole collection. By using Scheme-1 with the frequency patterns of the 10 qualitative traits fixed as per the square root-proportion method, the SDI values of these descriptors except growth habit were significantly

Table 6. Diversity measure[$] for descriptors in the core samples obtained through the schemes 1, 2, 5 and 6

Descriptor	Whole collection	No Stratification			With Stratification			Pre-assigned SDI[$]		
		SRS#	Scheme-1	Scheme-5	SRS–	Scheme-2	SRS-LF#	Scheme-6	Scheme –1&2	Scheme –5&6
Margin of lower stem leaf	0.322	0.321	0.595**	0.740**	0.384	0.553**	0.410**	0.712	0.751	0.973
Texture of upper leaves	0.625	0.626	0.879**	0.882**	0.701	0.853**	0.727**	0.852	0.835	0.912
Spines on upper stem leaves	0.521	0.518	0.835**	0.862**	0.655	0.809**	0.703**	0.852	0.845	0.983
Location of spines on OIB	0.249	0.245	0.642**	0.859**	0.359	0.559**	0.403**	0.779	0.686	0.844
No. of spines on OIB	0.535	0.534	0.963**	0.965**	0.718	0.942**	0.791**	0.974	0.870	0.988
Length of spines on OIB	0.607	0.605	0.945**	0.944**	0.781	0.925**	0.834**	0.941	0.890	0.990
Bracts enclosing head	0.319	0.323	0.771**	0.884**	0.414	0.677**	0.450**	0.840	0.722	0.968
Growth habit	0.893	0.893	0.897	0.899	0.927	0.881	0.908	0.892	0.962	0.995
Pollen production	0.822	0.821	0.983**	0.965**	0.930	0.984*	0.967	0.974	0.954	0.995
Hull thickness	0.638	0.636	0.850**	0.807**	0.737	0.863*	0.762	0.850	0.889	0.990
Days to physiological maturity	0.650	0.648	0.566**	0.597*	0.671	0.525**	0.556**	0.759	0.676	0.676
No. of primary branches	0.723	0.726	0.804**	0.705	0.717	0.780**	0.660*	0.730	0.846	0.846
No. of capitula/plant	0.684	0.685	0.857**	0.742*	0.723	0.841**	0.705	0.734	0.887	0.887
Inter-node length	0.559	0.563	0.717**	0.607	0.622	0.693*	0.608	0.719	0.756	0.756
Main capitula diameter	0.556	0.555	0.642**	0.581	0.606	0.643	0.580	0.701	0.742	0.742
Geographical origin	0.416	0.418	0.805**	0.773**	0.863	0.878	0.981	0.844	0.863	0.981
No. of accessions	3250	420	420	420	420	420	420	420	–	–

$: Shannon Diversity Index in standardized form

#: SRS– Simple Random Sampling; SRS-SQP: Stratified Random Sampling with Square root-proportion allocation for geographical regions;

SRS-LF: Stratified Random Sampling with Log-Frequency allocation for geographical regions

*, **: SDI significantly different from the corresponding SRS values at 5% and 1% levels, respectively.

higher than those obtained through SRS or the whole collection. For the descriptors margin of lower stem leaves, location of spines on OIB and bracts enclosing head, the diversity level was quite low in the whole collection but Scheme 1 yielded a core sample with significantly higher diversity than the whole collection. The diversity levels for the quantitative descriptors were also significantly higher than those of SRS, except days to physiological maturity for which the diversity in the core sample was significantly lesser under Scheme 1. Nevertheless the diversity in the core sample by Scheme-1 was somewhat short of the pre-assigned levels for some of the descriptors as it was indicated earlier that this core sample was reduced to the required size from the first stage core collection of 510 accessions. The results for Scheme 5 indicated similar trends as for Scheme-1 and in addition the diversity levels for the descriptors were much higher in case of qualitative attributes. For both these schemes, the geographical diversity in the core sample was significantly higher than the whole collection or as that of core sample through SRS. The schemes 2 and 6 wherein stratification of the accessions was considered, the SDI values for the qualitative descriptors (except 'growth habit') were significantly higher than those obtained through stratified random sampling where the accessions in the strata were assigned as per the square root-proportion method or log-frequency method. The comparison of the SDI values of quantitative traits for these two schemes against stratified random sampling indicated that days to physiological maturity had significant lower level of diversity for both these schemes. In general, core samples obtained through schemes 5 and 6 (with stratification of accessions as per geographical origin) had lower levels of diversity for the descriptors than those obtained through schemes 1 and 2. But the stratification obviously increased the diversity level of the accessions from different geographical regions.

In Table 7, the diversity index for the descriptors is presented for core samples obtained through schemes 3, 4, 7 and 8 (core sample size = 325) and for the core samples obtained through simple or stratified random sampling. In these schemes only the distribution patterns for 10 qualitative traits were pre-assigned. The diversity levels for the quantitative traits have also been presented in this table for the sake of comparison. The trends were similar to those of schemes 1, 2, 5 and 6, except that the quantitative descriptors had the same level of diversity as that obtained under simple random sampling or stratified random samples. Also the diversity levels in the core samples obtained through the schemes 3, 4, 7 and 8 were higher for location of spines on IOB and bracts enclosing head which had low diversity in the whole collection. For these schemes also the realized diversity levels were short of the target in view of the fact that the first stage core samples were reduced to the required size. For the schemes which included stratification of accessions as their geographical origins (2, 6, 4 and 8), the SDI values were somewhat less than the schemes without stratification. The results also indicated that for the descriptors that had high level of diversity in the whole collection, not much improvement could be obtained in the core samples. But for the descriptors with low to moderate levels of diversity in the whole collection, the proposed method enhanced their diversity significantly in the core sample.

Review of earlier work so far in this direction indicates a general rule for establishing a core sample that is as follows. Make use of passport, characterization and other relevant information to first identify groups of accessions; then select a sample from each group either randomly or by purposive sampling. The sampling fraction from the different groups might however vary depending on the data. The method suggested by Noirot *et al.*, (1996) markedly deviated from this approach. They demonstrated that by using principal component analysis (PCA) on a set of quantitative descriptors, the sampling strategy could be changed by selecting accessions with the largest deviations from the centroid of the whole collection or the individual strata to constitute the core sample. Though the PCA method was not specific about qualitative variables, it resulted in core samples that had almost the same or slightly higher amount of diversity for several qualitative attributes as that in the whole collection (Mahajan *et al.*, 1996, Balakrishnan *et al.*, 2000 and Balakrishnan and Suresh, 2000). However, it would be a great advantage from the users' point of view if a core sample could be obtained with pre-assigned levels of diversity for several characteristics that are both multi-state and continuous for a fixed sample size. For this purpose we need to specify the density distribution of the core sample, which automatically fixes the density distribution of the remaining accessions. Then the problem becomes one of assigning an accession with a given set of attribute values to either of the two groups based on some objective criterion. Wallace & Boulton (1968) using the information theory concepts have shown that this problem is equivalent to a classification scheme that minimizes an information measure with the following properties for the groups:

Table 7. Diversity measure[$] for descriptors in the core samples obtained through the schemes 3,4, 7 and 8

Descriptor collection	No Stratification Whole	With Stratification		Pre-assigned SDI[$]					
		SRS[#]	Scheme-3	Scheme-7	SRS-SQP[#]	Scheme-4	SRS-LF#	Scheme-8	Schm-3&4	Schm-7&8
Margin of lower stem leaf	0.322	0.329	0.567**	0.626**	0.390	0.599**	0.408	0.627**	0.751	0.973
Texture of upper leaves	0.625	0.627	0.803**	0.908**	0.697	0.896**	0.732	0.911**	0.835	0.912
Spines on upper stem leaves	0.521	0.529	0.788**	0.780**	0.653	0.786**	0.706	0.776	0.845	0.983
Location of spines on OIB	0.249	0.249	0.873**	0.946**	0.357	0.767**	0.404	0.893**	0.686	0.844
No. of spines on OIB	0.535	0.532	0.843**	0.908**	0.722	0.951**	0.798	0.950**	0.870	0.988
Length of spines on OIB	0.607	0.609	0.809**	0.861**	0.780	*0.869*	0.840	0.892	0.890	0.990
Bracts enclosing head	0.319	0.322	0.981**	0.879**	0.424	0.876**	0.455	0.876**	0.722	0.968
Growth habit	0.893	0.892	0.902	0.887	0.929	0.853	0.908	0.859	0.962	0.995
Pollen production	0.822	0.815	0.904*	0.939**	0.933	0.957	0.964	0.951	0.954	0.995
Hull thickness	0.638	0.638	0.722	0.779*	0.723	0.805	0.756	0.805	0.889	0.990
Days to physiological maturity	0.650	0.654	0.659	0.670	0.675	0.649	0.643	0.651	0.676	0.676
No. of primary branches	0.723	0.724	0.699	0.720	0.717	0.694	0.704	0.709	0.846	0.846
No. of capitula/plant	0.684	0.686	0.684	0.707	0.723	0.693	0.733	0.707	0.887	0.887
Inter-node length	0.559	0.559	0.679	0.601	0.623	0.621	0.657	0.620	0.756	0.756
Main capitula diameter	0.556	0.560	0.640*	0.614	0.601	0.630	0.625	0.630	0.742	0.742
Geographical origin	0.416	0.415	0.713**	0.868**	0.863	0.868	0.981	0.849	0.863	0.981
No. of accessions	3250	325	325	325	325	325	325	325	-	-

$: Shannon Diversity Index in standardized form

#: SRS – Simple Random Sampling;
SRS-SQP: Stratified Random Sampling with Square root-proportion allocation for geographical regions;
SRS-LF: Stratified Random Sampling with Log-Frequency allocation for geographical regions

*, **: SDI significantly different from the corresponding SRS values at 5% and 1% levels, respectively.

a) A probability distribution function in measurement space is provided for each group (*viz.*, the core and non-core sample groups in the current context).
b) The parameters describing the probability distribution for a group are assigned values that are essentially maximum likelihood estimates based on the individuals assigned to that group.
c) Each individual is assigned to that group which has the highest density in the neighbourhood of the thing in the measurement space.

The information measure F(k,t) specified in equation (6) is an ideal criterion as it is derivable using the existing data for the accessions in the whole collection. It is based on comparisons between an individual on the one hand and a group on the other, and uses the well defined concept of probability that a member of a group of known distribution would be found to have certain measurement attributes. The properties of the core samples that were obtained in the present investigation using various schemes of pre-assigning the density distribution of the core sample clearly demonstrated that it would be possible to substantially increase the diversity of attributes which are low or moderate in the whole collection. Probably this would not have been possible by the other methods available so far. The frequency patterns of the final core sample size were not close to the pre-assigned ones, and the deviations that we observed could be mainly attributable to the inclusion of several qualitative and quantitative descriptors each having its own independent density distribution. The increased diversities of several attributes from the smaller core samples obtained from Schemes-3, 4, 7 and 8 clearly indicated the potentials of the proposed method as a new and powerful technique for constituting core samples.

The proposed method could be easily adapted to a variety of options with regard to grouping or stratification of the accessions. For the present study we have taken into consideration the geographical origins of the accessions as the grouping criterion. The core samples obtained using the stratification scheme had better diversity than the whole collection for the attribute under consideration, *viz.*, the geographical origin.

For pre-assigning the descriptor state frequencies for the core sample we have considered the square root-frequency proportion method and the log-frequency method as they were found to be useful frequency transformation methods. In fact, we could use a general class of frequency transformation of the form: $q_i = p_i^{\alpha}/(\sum_{j=1}^{s} p_j^{\alpha})$, s being the number of descriptor states and α is some constant greater than zero (Theil, 1972). The square root-frequency proportion method used in this investigation corresponds to a value of $\alpha = 0.5$.

Any value of a less than 1 will increase the SDI value in the core sample as compared to that of the whole collection, but SDI would be much higher if a is less than 0.5. The log-frequency transformation suggested by Brown (1989) is one such method but it generally reduces the frequency levels of predominant attributes rather sharply. A value of a > 0.5 towards 1 will correspondingly reduce the diversity in the core sample than that when a < 0.5. The proportional method of frequency transformation corresponds to $\alpha = 1$. But this method of pre-assigning the frequency patterns for all the descriptors in the core sample to the same level as that in the whole collection is just equivalent to simple random sampling. Also, it is not useful for obtaining a core sample using the proposed information measure as it would result in F(k,1) > F(k,2) for all accessions and no accessions could be allocated to the core sample. But it is possible to adopt different proportions of q_i for different descriptors by keeping different values of α such that $\sum q_i = 1$ for each descriptor. For example, we can pre-assign the sampling fraction from different geographical regions using log-frequency transformation and pre-assign the density distribution for a qualitative descriptor by fixing $\alpha = 0.3$ or 0.4. In the present study we have fixed the frequency patterns for the quantitative descriptors based on a different criterion.

It should be noted that if an optimum core sample size could be decided for the set of pre-assigned density distributions for various descriptors, then the large deviations in the first stage core samples from the pre-assigned ones could be reduced to a great extent. The frequency patterns in the core sample and the core sample sizes were considered mainly to illustrate the advantages of the new procedure. Further research is needed for optimizing the core sample size for a given set of frequency patterns or *vice versa.* The proposed method has one major advantage that any new accession that is added to the genetic resource may be assigned to the core sample group or the non-core sample group by computing the appropriate information statistic F(k,t) that depends on the measurements of the new accession on several descriptors. It is also possible to have more than one working core collection from the same genetic resource by using different pre-assigned frequency profiles for different sets of descriptors.

Conclusions

In the present investigation, a new approach for obtaining a core sample from any large germplasm collection has been proposed. The problem has been treated as one of objectively assigning an accession to the core sample group or the reserve collection which have pre-assigned sample sizes and density distributions for various attributes. For this, an information measure that depends on the probability of obtaining an accession with a given set of measurements or attributes in the vicinity of the presumed composite density of either of the two groups has been used. The new method also incorporates grouping of the accessions into strata based on some criterion like geographical origins. The results of the present investigation have clearly demonstrated the potential use of this methodology for obtaining core samples with desirable combination of distribution profiles of various characteristics.

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Response to Selection under Sib-mated and Selfing Progeny in Wheat (*Triticum aestivum* L.)

Tejbir Singh and SB Singh
Department of Agricultural Botany, Kisan PG College, Simbhaoli-245207, Uttar Pradesh

The relative efficiency of the biparental mating system and selfing series was evaluated in terms of response to selection for grain yield following phenotypic and geno-phenotypic selection procedures in two crosses of wheat. The variability and the response to selection for grain yield was more under biparental mating system than under selfing series. The realized response due to the first cycle of selection and predicted response due to second cycle of selection indicated that geno-phenotypic procedure of selection was more efficient than phenotypic selection procedure. An index comprising grain yield, tiller number and biological yield could be used for selecting high yielding genotypes.

Key Words: Phenotypic and Geno-phenotypic Selection, Predicted and Realized Response, Sib-mating/ Biparental Mating

The occurrence of recombinants in the traditional method of breeding is not only restricted by the limited recombination potential but also by the possible character associations resulting due to linkage and/or pleiotropism (Anderson, 1939; Dempster, 1949). The consequences of linkage and pleiotropism in relation to the potential breeding value of the material are different and are of critical importance. Hanson (1959) and Miller and Rawlings (1967) proposed that intermating in segregating generation might tend to break up linkages. Further this approach is also expected to retain greater variability for several cycles of selection, to elevate the population mean and to improve the chances of assembling the maximum number of potentially important genes leading to the isolation of stable and widely adapted genotype(s). A few studies that have been conducted on the vital aspect of usefulness of various mating systems and selection procedures have yielded divergent results. The present study was conducted with a view to gain information on the predicted and realized genetic gain under two mating systems and selection procedures for yield improvement in wheat.

Materials and Methods

The materials for the present study comprised two F_2 populations *viz.* WH 542/Raj 3077 (cross I) and WH 147/HD 2329 (cross II). In each of the F_2 populations, 120 random plants were selfed and crossed in pairs (biparental mating) to obtain 120 F_3 families and 60 $BIPF_1$'s hybrids, respectively. The parental lines, 120 F_3 families and 60 $BIPF_1$'s in each of the cross I and cross II were evaluated in three and two replications, respectively. Each entry in a replication was assigned to a single row plot of 2 m length with a distance of 30 cm and 10 cm between rows and plants, respectively. Data was recorded on all plants except the border plants in each plot on 10 characters (listed in Table 2) in both the crosses.

In both the segregating generations (F_3, $BIPF_1$'s) nature selfing was allowed and selection for grain yield was applied in two ways: (i) Phenotypic selection (P); based on individual plant merit, 5% top yielding plants were selected, (ii) geno-phenotypic selection (GP); based on both families and individual plant merit two highest yielding plants from each of the 50% top yielding families were selected maintaining a 5% selection intensity. By following this procedure two parents, $BIPF_2$ and F_4 progenies were planted in a randomized block design experiment with three replications at research farm, Kisan P.G. College, Simbhaoli (Ghaziabad). Data on the five random plants were recorded on the above 10 characters.

Plot means were used for different statistical analyses. The response to selection was calculated as a difference between the mean relative value of the offspring of selected plants and parental generation before selection. Heritability (broad sense) and predicted response for the directly selected and correlated traits were calculated according to Burton and DeVane (1953) and Johnson *et al.* (1995), respectively. The phenotypic correlation coefficients were estimated according to Al-Jibouri *et al.*, (1958).

Results and Discussion

The parental lines involved in the F_2 populations differed significantly for most of the characters suggesting genetic

diversity among parents. The mean values for grain yield and several other characters of $BIPF_1$ populations deviated significantly from mean values of their respective F_3 population (Table 1), although the two base populations (F_3 and $BIPF_1$) received the same input of gametes. The deviation in the mean values may thus be attributed to dominance deviations and epistatic interactions in $BIPF_1$ populations (Mather and Jinks, 1971).

Table 1. Mean grain yield of various base and selected populations of cross I and cross II

Population	Cross I	Cross II
F_3	9.45	14.40
$BIPF_1$	13.60^{aa}	17.65^{aa}
F_4P	11.35	15.23
$BIPF_2P$	19.56^{bb}	18.90^{bb}
F_4GP	13.38	16.78
$BIPF_2GP$	21.97^{cc}	20.90^{cc}

P = Phenotypic; GP = Geno-phenotypic; aa = F_3 vs $BIPF_1$; bb = F_4P vs $BIPF_2P$; cc = F_4GP vs $BIPF_2GP$; means significantly higher at P = 0.01 level

Due to first cycle of selection the predicted response (as per cent mean of checks) for grain yield and the correlated responses for other nine characters, except plant height (cross I and cross II), tiller number and biological yield (cross I) and spikelets/spike (cross II) were relatively high in $BIPF_1$ populations than in the F_3 populations (Table 2). The predicted response to selection for grain yield and correlated responses for other nine characters were, however, lower than the realized response to selection (Table 2). This discrepancy in the predicted and realized response due to first cycle of selection may be attributed to the biased estimates of genotypic variance and heritability and also some extent to g × e interaction. Further, it was proposed by Robertson (1977) and Nishida and Abe (1974) that positive skewness of genotypic distribution and negative skewness of environmental distribution for a character, the realized response to selection is expected to be high than the predicted response.

The average realized selection response due to first cycle of the selection was greater under biparental mating than under selfing series which may be attributed to increased genetic variability due to recombination that may have taken place following intermating and to relatively high inbreeding depression in selfing series.

Table 2. Predicted and realized response (% mean of checks) for 10 characters in base (F_3 and $BIPF_1$) and selected (F_4 and $BIPF_2$) population in cross I and II

Sl.No.	Characters	Base Population		Selected Population			
		F_3	$BIPF_1$	F_4		$BIPF_2$	
				P	GP	P	GP
Cross I							
1.	Grain yield	11.83	28.18	20.11	41.594	43.82	61.54
2	Days to heading	-0.02	0.69	-1.19	-0.63	2.63	3.12
3.	Days to maturity	-0.08	0.71	-6.09	-5.29	-7.64	-9.55
4	Plant height	0.67	-0.36	19.46	18.87	15.45	10.36
5.	Tiller number	2.85	1.32	40.98	28.06	14.34	51.71
6	Spikelets/spike	-0.49	0.56	9.53	11.67	4.13	5.29
7.	Grain/spike	0.05	0.17	-6.47	2.11	12.04	14.74
8.	100-grain weight	0.60	0.89	14.21	9.95	35.28	35.83
9.	Biological yield	2.46	0.77	-16.73	4.75	11.81	27.37
10.	Harvest index	0.00	1.29	44.22	35.17	28.67	26.86
Cross II							
1.	Grain yield	9.88	32.34	5.76	16.53	7.08	18.41
2.	Days to heading	0.02	0.67	-6.15	-8.51	4.38	3.03
3.	Days to maturity	-0.14	0.23	-7.61	-8.62	-10.92	-11.14
4.	Plant height	0.59	0.25	0.28	2.81	0.26	-0.74
5	Tiller number	0.31	5.01	17.06	21.91	13.40	38.01
6	Spikelets/spike	-0.78	0.56	2.61	6.75	0.50	4.89
7	grains/spike	-0.52	1.02	-1.25	-4.39	11.80	4.99
8.	100-grain weight	0.35	0.59	1.17	4.24	13.15	14.21
9.	Biological yield	1.39	4.58	4.37	8.54	4.52	6.68
10.	Harvest index	-0.18	0. 85	1.33	7.37	2.47	11.02

P = Phenotypic; GP = Geno-phenotypic

In agreement with the present results several earlier workers Balyan and Verma (1985), Kaushik *et al.*, (1996) and Salvagiotti and Maich (1999) also recommended the use of intermating in early segregating generations in wheat to break undesirable linkages and to retain greater variability for several cycles of selection.

The mean grain yield of the $BIPF_2$ population was also significantly higher the mean grain yield of respective F_4 populations in both the crosses. Moreover, the frequency distribution pattern of families and plants, on the basis of grain yield per plant, showed that a number of families and plants were grouped in the highest yielding class in $BIPF_2$ population against one in F_4 population.

The average predicted selection response for grain yield due to second cycle of selection was 49.29% and 45.06% in $BIPF_2$ populations than in F_4 populations of cross I and cross II, respectively (Table 3). The superiority of predicted response to selection in $BIPF_2$ over F_4 populations may partly be attributed to reduced variability and higher inbreeding depression in F_4 populations due to two cycles of inbreeding under selfing series as compared to only one cycle of inbreeding under biparental mating system.

The results also showed that owing to the positively correlated response of tiller number, biological yield and harvest index towards increased grain yield selection,

Table 3. Predicted response for second cycle of selection (% mean of checks) for 10 characters in selected (F_4 and $BIPF_2$) populations of cross I and II

Character	Cross I				Cross II			
	F_4		$BIPF_2$		F_4		$BIPF_2$	
	P	GP	P	GP	P	GP	P	GP
Grain yield	18.23	38.17	77.12	77.80	13.19	3.61	53.85	53.06
Days to heading	-070	0.04	-0.85	-0.89	0.23	0.48	0.78	0.36
Days to maturity	-0.42	-0.17	1.36	0.96	-0.29	-0.19	1.47	-0.46
Plant height	-0.62	-0.37	-0.76	-0.32	0.97	-0.94	0.19	-0.42
Tiller number	1.55	2.82	6.05	5.81	1.71	0.49	1.82	6.39
Spikelets/spike	-0.52	-0.24	-1.63	-1.90	–0.74	0.58	0.73	-0.48
Grains/spike	-0.92	1.12	-1.25	-1.62	-0.10	0.03	1.91	0.58
100-grain weight	-0.37	0.65	3.31	1.27	0.17	-1.64	2.15	1.14
Biological yield	1.63	3.47	6.70	6.77	1.65	0.38	6.72	6.65
Harvest index	-019	0.65	5.15	4.54	0.19	0.31	4.68	4.92

P = phenotypic; GP = Geno-phenotypic

these traits become important yield determining characters in various selected population (Tables 2 and 3). These characters were also significantly and positively associated with grain yield in base and selected populations (Table 4).

In the past also, the improvement of wheat yields were realized primarily because of the high harvest index of semi-dwarf wheats compared to old tall wheats. Biological yield has a high economic value, yet selection for it is not generally made. Sharma (1992) reported that high biomass yielding wheat genotypes are preferred by the farmers in the developing countries because such genotypes produce a high grain yield as well as high yield of non-grain plant parts.

It may be concluded that the efficiency of selection using P and GP procedures under selfing series and biparental mating system were highly effective in improving grain yield. However, for the second cycle of selection on the basis of predicted response the population derived through biparental mating system was expected to be superior in terms of yield improvement. Further, under biparental mating system GP selection procedure seemed to be more efficient than P selection procedure. The superiority of GP selection procedure emanates from the fact that selection of plants in this procedure is based on their phenotypic values as well as genetic worth of their parental families. In agreement with the present result, it was also argued that the selection of best plants within selected families appears to be effective means of improving yield as compared to selection of best plant (Thakare and Qualset, 1978).

Table 4. Phenotypic correlation coefficients between grain yield and other characters in different populations under selfing and biparental mating system in cross I and cross II

Character Combination	F_3	$BIPF_1$	F_4		$BIPF_2$	
			P	GP	P	GP
CROSS I						
Grain yield *vs.*						
-Days to heading	-0.007	0.032	-0.559**	0.051	-0.124	-0.127
-Days to maturity	-0.090	0.117	-0.081	-0.062	0.199	0.135
-Plant height	0.125	-0.044	-0.083	-0.047	-0.114	-0.047
-Tiller number	0.904**	0.919**	0.952**	0.799**	0.883**	0.843**
-Spikelets/spike	-0.093	0.056.	-0.157	0.007	-0.237	-0.274
-Grains/spike	-0.082	0.019	-0.114	0.179	-0.179	-0.234
-100-grain weight	0.032	-0.192	-0.035	0.085	0.488*	0.188
-Biological yield	0.930**	0.968**	0.974**	0.960**	0.987**	0.987**
-Harvest Index	0.078	0.317*	0.007	0.172	0.748**	0.661**
CROSS II						
Grain yield *vs.*						
-Days to heading	0.038	0.055	0.068	0.130	0.115	0.054
-Days to maturity	0.001	-0.081	0.056	-0.085	0.216	-0.065
-Plant height	0.137	0.023	0.202	-0.149	0.027	-0.063
-Tiller number	0.033	0.890**	0.951**	0.872**	0.266	0.947**
-Spikelets/spike	0.069	0.009	-0.084	-0.022	0.106	-0.072
-Grains/spike	-0050	0.174	0.038	-0.008	0.279	0.086
-100-grain weight	-0.226*	0.015	0.066	-0.231	0.312*	0.169
-Biological yield	0.974**	0.848**	0.971**	0.974**	0.978**	0.985**
Harvest Index	0.174*	0.139	0.115	0.202	0.688**	0.735**

P = Phenotypic; GP = Geno-phenotypic; *,** Significant at P = 0.01 levels, respectively.

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Genetic Relationship of Growth and Developmental Traits with Seed Yield in Dwarf Field Pea

Payal Vaishnav and RL Pandey
Department of Plant Breeding and Genetics, Indira Gandhi Agricultural University, Raipur-492012, Chhattisgarh

Twenty-five genotypes of diverse origin of dwarf pea (*Pisum sativum*, L.) were analysed for their yield and various yield contributing characters. Correlation co-efficient analysis revealed that seed yield was positively associated with days to 50% flowering, first pod bearing node, pods/cluster, pod length, seeds/pod and harvest index. Path co-efficient analysis revealed that harvest index, biological yield, pod number, pods/cluster and pod length seem to be the major yield contributing traits in dwarf field pea.

Key Words: Correlation Co-efficient; Field Pea; Path Analysis

Seed yield is not a unitary character but depends on the development of various plant characters. Contribution of each character towards increase in seed yield varies among the crops. Seed yield primarily depends upon the magnitude and the nature of genetic variability present in the population. The knowledge of association of seed yield with its component traits, helps in achieving success in a breeding programme. Therefore, an experiment was undertaken to determine the direct and indirect association among yield traits through path co-efficient analysis in peas.

Materials and Methods

Twenty-five genotypes of dwarf field pea were grown in Randomized Complete Block Design with three replications during *rabi* 1999-2000, at Indira Gandhi Agricultural University, Raipur. Each genotype was represented by five rows of 4 m, row to row distance was 30 cm and seeds were sown at 10 cm space within each row. Observations on days to 50% flowering and maturity were recorded on plot basis. Plant height, number of branches, number of clusters, first pod bearing node, pod bearing length, % seed setting, pods/cluster, pods/plant, pod length, seeds/pod, 100-seed weight, biological yield, seed yield/plant and harvest index were recorded on five competitive plants in each genotype. Correlation co-efficient among the variables and path co-efficient analysis were computed by using the formulae suggested by Falconer (1964) and Dewey and Lu (1959) respectively.

Results and Discussion

Correlation Co-efficient Analysis

Estimates of genotypic and phenotypic correlation co-efficient showed that genotypic correlation co-efficients in general were higher in magnitude than phenotypic ones for most of the traits (Table 1) indicating, least influence of environmental factors in the expression of these traits.

Genotypic correlations revealed that days to 50% flowering had shown significantly positive correlation with maturity, number of branches, first pod bearing node, pods/cluster and seed yield/plant while it had significantly negative association with clusters/plant. These results are in agreement with Chandirakala and Raveendran (1998). Days to maturity exhibited significant positive association with branches/plant, % seed setting and pods/cluster. These results are in conformity with the result of Pratap *et al.*, (1995). Significant positive association of plant height was recorded with first pod bearing node, seeds/pod and biological yield while it was negative with pods/plant, clusters/plant and harvest index indicating that taller plant adversely affected these traits. Similar findings were also noted by Dev and Rastogi (1999). It is obvious to mention here that branches in field pea had no association with any character and showed independent behaviour.

Clusters/plant had positive and significant association with pod bearing length, pods/plant and harvest index. This clearly indicates that higher number of clusters/plant with longer pod bearing length and higher harvest index will be useful increasing the number of pods and harvest index in dwarf field pea. In contrast first pod bearing node, pod length, seeds/pod and biological yield were negatively associated with clusters/plant. First pod bearing node was positively correlated with pods/cluster, pod length, seeds/pod, biological yield and seed yield/plant. While it had negative association with pod bearing length, % seed setting and pods/plant. The

Table 1. Correlation-coefficient of yield components in dwarf field pea

Characters		Days to 50% flowering	Days to maturity	Plant height	Branches/ plant	Cluster/ plant	First pod bearing node	Pod bearing length	Seed setting per cent	Pods/ cluster	Pods/ plant	Pod length	Seeds/ pod	100-seed wt	Biological yield/ plant	Seed yield/ plant	Harvest index
Days to 50%	G		0.793**	0.213**	0.364**	-0.255*	0.455**	-0.190	-0.031	0.498**	-0.189	0.193	-0.034	-0.129	0.112	0.471**	0.177
Flowering	P		0.795**	0.212	0.163	-0.249*	0.441**	-0.189	-0.027	0.461**	-0.184	0.186	-0.031	-0.121	0.106	0.353**	0.157
Days to	G			0.204	0.503**	-0.015	0.175	-0.043	0.310**	0.278**	-0.019	0.119	-0.165	-0.200	-0.054	0.213	0.165
maturity	P			0.202	0.222	-0.019	0.162	-0.042	0.340**	0.252**	-0.024	0.118	-0.154	-0.187	-0.058	0.152	0.143
Plant height	G				0.093	-0.241*	0.252*	0.273	-0.057	-0.117	-0.308**	0.134	0.242*	-0.066	0.497**	-0.190	-.514**
	P				0.037	0.238*	0.247*	0.266	-0.052	-0.103	-0.297*	0.127	0.228	0.069	0.487**	-0.147	-.466**
Branches/	G					-0.032	0.025	-0.187	-0.097	0.204	0.028	0.219	0.057	-0.141	0.161	0.159	-0.111
plant	P					0.061	0.000	-0.096	-0.099	0.116	0.066	0.104	0.065	-0.036	0.154	0.152	-0.040
Clusters/	G						-0.387**	0.403**	-0.031	-0.153	0.981**	-0.253*	-0.272*	0.033	-0.432**	-0.266*	0.231*
plant	P						-0.370**	0.395**	-0.032	-0.135	0.933**	-0.235**	-0.232**	0.042	-0.410**	-0.201	0.204
First pod	G							-0.312**	-0.261*	0.464**	-0.285*	0.538**	0.561**	-0.173	0.397**	0.734**	0.120
Bearing node	P							-0.310**	-0.251**	0.435**	-0.267*	0.505**	0.523**	-0.171	0.382**	0.562**	0.117
Pod Bearing	G								-0.166	0.036	0.334**	-0.266*	-0.224	-0.277*	-0.136	-0.372**	-0.081
Length	P								-0.164	-0.033	0.314**	-0.249*	-0.208	-0.265*	-0.133	-0.288*	-0.076
% seed setting	G									-0.094	-0.097	0.045	-0.073	-0.124	-0.152	-0.211*	-0.033
	P									-0.089	-0.090	0.046	-0.068	-0.116	-0.155	-0.123	-0.001
Pods/cluster	G										0.050	-0.063	-0.110	-0.518**	0.373**	0.440**	-0.091
	P										0.080	-0.058	-0.096	-0.476**	0.339**	0.347**	-0.054
Pods/plant	G											-0.323**	-0.293*	-0.021	-0.348**	-0.298*	0.166
	P											-0.302**	-0.265*	0.001	-0.315**	-0.140	0.157
Pod length	G												0.694**	0.010	0.098	0.766**	0.398
	P												0.621**	-0.028	0.095	0.525**	0.322
Seeds/pod	G													0.107	0.232*	0.517**	0.117
	P													0.097	0.220	0.376**	0.101
100-seed	G														-0.141	-0.251*	-0.015
weight	P														-0.133	-0.163	0.003
Biological	G															0.058	-.803**
Yield/plant	P															0.051	-.736**
Seed yield/	G																0.528**
plant	P																0.616**

G = Genotypic correlations, P = Phenotypic correlations, * = Significant at 5%, ** = Significant at 1%

observation are in confirmity with the results of Singh and Joshi (1982).

Pod bearing length showed significant positive association with pods/plant but negative with pod length, seed index and seed yield. Similarly, seed setting was negatively correlated with seed yield/ plant. Pods/cluster had significant positive association with biological yield and seed yield/plant However, it was negatively correlated with 100-seed weight. This finding is similar with Sharma *et al.*, (1997). Pods/plant was negative and significantly correlated with pod length, seeds/pod, biological yield and seed yield. Pods/plant is a major yield contributing character its negative correlation with seed yield might be affected by any indirect contributing trait. Pod length and seeds per pod were positively associated themselves and with seed yield/plant. Seeds/pod was also positively associated with biological and grain yield. Similarly, biological yield had negative and significant correlation with harvest index, which are in agreement with earlier findings of Pratap *et al.*, (1995).

It may be concluded from the present findings that seed yields was positively associated with days to 50% flowering, first pod bearing node/cluster, pod length, seeds/pod and harvest index.

Path Co-efficient Analysis

In present investigation path co-efficient analysis was carried out by considering seed yield as dependent and rest of the traits as independent variables. Data are presented in Table 2.

Harvest index had the highest direct affect on grain yield. It also had high positive correlation. Hence, direct selection for higher index in dwarf field pea will lead genetic grain in seed yield. Other important attributes positively contributed towards grain yield were biological yield, pods/plant, pods/cluster and pod length. Cluster/ plant also had high direct effects but in negative direction. Its correlation with grain yield, might be due to negative indirect effect via biological yield. These findings are in agreement with Chandirakala and Raveendra (1998) Pratap *et al.*, (1995).

Table 2. Path coefficient of seed yield contributing characters in dwarf field pea

Characters	Days to 50% flowering	Days to to maturity	Plant height	Branches/ plant	Cluster/ plant	First pod bearing node	Pod bearing length	Seed setting per cent	Pods/ cluster	Pods/ plant	Pods/ length	Seeds/ pod	100-seed wt	Biological yield/ plant	Harvest index	Genetic Correlation with seed yield/plant
Days to 50% flowering	**-0.001**	-0.009	0.010	0.011	0.095	-0.055	0.016	0.001	0.095	-0.058	0.033	-0.033	0.005	0.014	0.227	0.471**
Days to maturity	-0.001	**-0.012**	0.009	0.016	0.006	-0.021	0.004	-0.011	0.053	-0.006	0.020	-0.013	0.008	-0.050	0.211	0.213
Plant height	0.000	-0.002	**0.045**	0.003	0.090	-0.036	-0.024	0.002	-0.022	-0.095	0.023	0.019	0.003	0.460	0.660	-0.190
Branches/plant	0.000	-0.006	0.004	**0.031**	0.012	-0.003	0.016	0.003	0.039	0.009	0.038	0.004	0.005	0.149	-0.142	0.159
Cluster/plant	0.000	0.000	-0.011	-0.001	**-0.372**	0.047	-0.035	0.001	-0.029	0.303	-0.043	-0.021	-0.001	-0.400	0.297	-0.266*
First pod bearing node	-0.001	-0.002	0.011	0.001	0.144	**-0.121**	0.027	0.009	0.089	-0.088	0.092	0.044	0.007	0.368	0.154	0.734**
Pod bearing length	0.000	0.000	0.012	-0.006	-0.150	0.038	**-0.087**	0.006	-0.007	0.103	-0.046	-0.018	0.011	-0.126	-0.104	-0.372*
%Seed setting	0.000	-0.004	-0.003	-0.003	0.011	0.032	0.014	**0.036**	-0.018	-0.030	0.008	-0.006	0.005	-0.141	-0.042	-0.211*
Pods/cluster	-0.001	-0.003	-0.005	0.06	0.057	-0.056	0.003	0.003	**0.191**	0.016	-0.011	-0.009	0.020	0.346	-0.117	0.440**
Pods/plant	0.000	0.000	-0.004	0.001	-0.365	0.034	-0.029	0.004	0.010	**0.309**	-0.055	-0.023	0.001	-0.322	0.213	0.238*
Pod length	0.000	-0.001	0.006	0.007	0.094	-0.065	0.023	-0.002	-0.012	-0.100	**0.171**	0.054	0.000	0.091	0.499	0.766**
Seeds/pod	0.000	0.002	0.011	0.002	0.101	-0.008	0.019	0.003	-0.021	-0.090	0.119	**0.078**	-0.004	0.215	0.151	0.517**
100-seed weight	0.000	0.002	-0.003	-0.004	-0.012	0.021	0.024	0.004	-0.099	-0.006	0.002	0.008	**-0.038**	-0.131	-0.019	-0.251*
Biological yield/plant	0.000	0.001	0.022	0.005	0.161	-0.048	0.012	0.005	0.071	-0.107	0.017	0.018	0.005	**0.927**	-1.031	0.058
Harvest index	0.000	-0.002	-0.023	-0.003	-0.086	-0.014	0.007	0.001	-0.017	0.051	0.067	0.009	0.001	-0.744	**1.238**	0.528**

Residual effect = 0.0.22; The underscored figures denote the direct effects

Some of the attributes indirectly contributed on grain yield like days to 50% flowering and days to maturity also had positive indirect effects on grain yield through harvest index. Plant height and branches/plant had positive indirect effects *via* biological yield. However, these traits had shown negative indirect contribution through harvest index, indicating that further increase in plant height may adversely affect the grain yield in dwarf pea. Clusters/plant had positive indirect contribution through pods/plant and harvest index but negatively contributed through biological yield. Again it is clear that breeder should not try to increase the biomass in dwarf pea. First pod bearing node exhibited high positive indirect contribution through biological yield but also had low two indirect effects via. harvest index and clusters/plant. Pod bearing length exhibited positive indirect contribution through pods/plant while negatively through cluster/plant and harvest index suggesting breeding efforts for closer pod bearing through shorter internodes. Pods/cluster had positive contribution through biological yield and significant positive correlation, indicating that proper attention should be given during selection. Pods/plant had positive contribution through harvest index but negative indirect effects through biological yield and clusters/plant. Whereas, pod length had high positive indirect contribution through harvest index. Biological yield had positive indirect contribution via clusters/plant very high negative indirect effect through harvest index indicating that more emphasis on economic yield is needed.

It is clear from the above findings that harvest index, biological yield, pod number, pods/cluster and pod length seem to be major yield contributing traits in dwarf field pea, hence selection for these traits may lead to improvement in grain yield.

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Studies on Improved Methods of Mushroom Germplasm Conservation

SK Singh, RN Verma, RC Upadhyay and MC Yadav
National Research Centre for Mushroom, Chambaghat, Solan-173213, Himachal Pradesh

To determine best method(s) of mushroom germplasm conservation, 11 edible mushroom strains belonging to six genera *viz., Agaricus, Pleurotus, Auricularia, Lentinula, Morchella* and *Volvariella* were preserved using different short-, medium- and long term storage methods. Of short term preservation methods the longest survival of all the test strains was recorded in the treatment wherein the test strains were preserved on wheat grain spawn in the test tubes at 4°C. Out of 12 medium-term storage methods, higher recoveries are being made from four treatments wherein test strains were preserved in either liquid paraffin at room temperature or 4°C or in glycerol (20%) at room temperature or in the form of mycelial disc or spawn multiplied on wheat grains. However, *Lentinula edodes* could not survive in liquid paraffin at room temperature. *Morechella esculenta* could survive for 1 year and 4 months as mycelial disc in liquid paraffin at 4°C and in glycerol (20%) at room temperature. *Volvariella volcacoa* is being recovered only from one treatment wherein the fungus was preserved as mycelial disc in liquid paraffin at room temperature. In long-term liquid nitrogen preservation methods higher recoveries from liquid nitrogen preservation are being made for more than 20 months.

Key words: ***Agaricus, Auricularia,*** **Conservation, Cryopreservation,** ***Lentinula,*** **Mushroom,** ***Morchella, Pleurotus,*** **Preservation,** ***Volvariella***

Successful mushroom production depends upon the proper maintenance of pure culture spawn that is capable of providing fruiting bodies of high productivity with excellent flavour, palatable texture, colour and is pest and disease resistant. Maintenance of vigour and genetic characteristics of pure mycelium is main objective of strain preservation. Further, strain improvement of cultivated mushrooms and domestication of wild mushroom demands a well-planned system for the maintenance, preservation and availability of genetic diversity (Chang and Miles, 1989). There are various methods of maintenance and preservation of mushroom culture and culture collection centre should adopt more than one method to preserve them. These mushrooms might be of academic, industrial, medicinal or of horticultural importance.

Since there is no satisfactory method to check and evaluate the quality of spawn by rapid on-the-spot examination, a method of preserving selected strains which have been thoroughly tested and proved desirable is of primary importance. If no degenerative changes were to take place during the preparation or maintenance of mushroom cultures and of spawn, then the preservation of mushroom cultures would be a relatively simple, routine process. Unfortunately, it is not true. Degeneration of culture, or of the spawn produced by the culture, refers to the loss of desired qualities by changes that result in such things as slow development, poor rate of survival and low level of productivity (Chang and Miles, 1989; Stadelman, 1986).

Frequent subculturing, storage under mineral water, lyophilization and preservation in ultra low temperatures in liquid nitrogen at (–140°C to –196°C) are known methods of preservation for spore brearing cultures of microbes (Buell *et al.,* 1947; Boeswinkel, 1976; Smith and Onions, 1983; Kirsop and Doyle, 1991). Mushrooms are invariable stored as mycelial culture because spores of heterothallic or secondary homothallic species are produced through a sexual process and have genetic difference and may not always result in fruiting (Petersen, 1995). Since mushrooms are stored as mycelial cultures and in absence of hardy structures like double walled spores, cultures becomes susceptible to sudden change in temperatures and pressure (Franks, 1981). Therefore, attempts were made to develop protocols for slow cooling and rapid thawing of mushroom mycelium.

Materials and Methods

To determine best method(s) of mushroom germplasm conversation, eleven edible mushrooms strains *viz., Agaricus bisporus* (strain S-11 and U-3), *Agaricus bitorquis* (NCB-13), *Pleurotus flabellatus* (P1-50), *P. sajor-caju* (P1-10-A), *P. ostreatus* (P1-20), *P. sapidus* (P1-40), *Auricularia polytricha* (OE-4), *Lentinula edodes* (OE-9), *Morchella esculenta* (ME-2) and *Volvariella volvacea* (OE-12) were preserved using different short, medium- and long-term storage methods.

Pure cultures of all the 11 strains were raised using tissue culture method. The cultures were conserved using different methods. For short-term storage methods, six

treatments on which pure cultures were raised on wheat extract agar (WEA) culture medium in test tubes (Singh, *et al.*, 2000) and preserved and T1 = room temperature, T2 = at 4°C, T3 = at –10°C, T4 = pure culture spawn raised on wheat grains in test tubes and stored at room temperatures, T5 = at 4°C and T6 = at –10°C, Observations on effect of short term preservation methods was recorded after every two months from the date of preservation.

For medium-term storage methods, pure cultures were first raised on wheat extract agar (WEA) mediums in petriplates and then 5 mm mycelial disc were cut and preserved in sterile distilled water (SDW), liguid paraffin (LP) or glycerol at room temperature, 4°C and –10°C. Twelve treatments were compared for per cent recovery of different mushroom strains at fixed interval of 4 months. Medium term storage treatments were as under:

T1 = Mycelial disc in SWD at room temp.
T2 = Mycelial disc in SWD at 4°C
T3 = Mycelial disc in SWD at room temp.
T4 = Mycelial disc in LP at 4°C.
T5 = Mycelial disc in glycerol (20%) at room temp.
T6 = On wheat grains in SDW at room temp.
T7 = On wheat grains in SDW at 4°C.
T8 = On wheat grains in LP at room temp.
T9 = On wheat grains in LP at 4°C.
T10 = On wheat grains in glycerol (20%) at 4°C.
T11 = On wheat grains in SDW at –10°C.
T12 = On wheat grains in glycerol at (20%) –10°C.

In long term preservation treatments, all the 11 test strains were preserved both as mycelial disc (5 mm) and fungus multiplied on wheat grain spawn and preserved in cryovials containing glycerol as cryoprotectant in liquid nitrogen at –196°C following the principle of slow cooling and rapid thawing. Cryovials were first placed for 4 h at –20°C and then for 6 h at –70°C in a mechanical freezer before plunging into liquid nitrogen at –196°C. Recoveries were made by placing the cryovials in water blanks each containing 50 ml SDW maintained at 37°C for rapid thawing. The cultures were retrieved after six months.

Results and Discussion

The results of short-term preservation are presented in Table 1. The data exhibits that the test mushroom strains survival for larger periods in the form of spawn as compared to preservation in the form of pure cultures raised on WEA medium. The longest survival of all the preserved strains for 1 year 8 months was recorded in the treatment T5 where in the test strains were preserved on wheat grain spawn in the test tubes at 4°C. The longer survival of mushroom mycelium on wheat grain spawn as compared to culture medium can be attributed to the fact that the mycelium invaded the soft wheat grains and remained concealed inside and nutrients were available for longer period whereas, culture medium dried upon prolonged storage. Whereas, prolonged storage at –10°C without cryopetectant could have destroyed the soft and tender mycelium.

The result of per cent recovery of different mushroom strain are presented vide Table 2. The data exhibits that the higher recovery of most of the strains is being made for more than 1 year and 8 months from treatment T3, T4, T5 and T9 wherein test strains were preserved in either liquid paraffin at room temp., 4°C or in glycerol (20%) at room temperature either in the form of mycelial disc or spawn multiplied on wheat grains. However, *Lentinula edodes* (OE-9) is being retrieved from only three treatments T4, T5 and T9. *Morchella esculenta* could survive up to 1 year 4 months in treatments T4 and T5. *Volvariella volvacea* (OE-12) was recovered from T3 treatment, wherein the fungus was preserved as mycelial disc in liquid paraffin at room temperature.

In medium-term storage higher recoveries in treatments T3, T4 T5 and T9 can be attributed to the fact that in liquid paraffin and glycerol the mycelial disc and mycelium on wheat grain spawn remained disconnected with atmospheric oxygen. Whereas in SDW, most of the test strains over-grew water surface and formed a mycelial mat which dried later.

The results of long-term liquid nitrogen preservation methods indicate higher recoveries from liquid nitrogen preservation in the form of wheat grain spawn as compared to mycelial disc preservation. All the test strains are being preserved satisfactorily in liquid nitrogen on wheat grain spawn for 1 year and 10 months (Table 3). The treatments with higher recoveries in liquid nitrogen on wheat grain spawn for 1 year and 10 months (Table 3). The treatments with higher recoveries in liquid nitrogen on wheat grain spawn can be attributed to the fact that the test fungi remained protected being concealed inside the grains and could have sustained slow cooling pressures as the test cultures were submerged in glycerol

Table 1. Effects of short-term preservation methods on survival of edible mushrooms

Mushroom Strain	Treatment	Per cent recovery		Period of survival	Remarks
		First recovery (%)	Last recovery (%)		
S-11	T_1	100	30	12	
	T_2	100	40	14	
	T_3	90	20	12	
	T_4	100	60	14	
	T_5	100	30	20	Survival Continued
	T_6	100	10	20	Survival continued
U_3	T_1	100	30	12	
	T_2	100	20	14	
	T_3	100	40	10	
	T_4	100	30	18	
	T_5	100	50	20	
	T_6	100	10	20	
NCB-13	T_1	100	30	12	
	T_2	100	50	14	
	T_3	80	20	10	
	T_4	100	30	18	
	T_5	100	50	20	Survival continued
	T_6	100	40	20	Survival continued
P1-20	T_1	100	30	12	
	T_2	100	20	14	
	T_3	90	40	8	Survival continued
	T_4	100	20	10	
	T_5	100	50	20	
	T_6	100	30	20	
P1-50	T_1	100	30	20	Survival continued
	T_2	100	10	14	
	T_3	80	20	10	
	T_4	100	20	16	
	T_5	100	50	20	
	T_6	100	20	20	Survival continued
P1-40	T_1	100	20	12	
	T_2	100	20	14	
	T_3	100	10	8	
	T_4	100	20	18	Survival continued
	T_5	100	50	20	
	T_6	100	80	20	
P1-10A	T_1	100	20	12	Survival continued
	T_2	100	30	14	
	T_3	100	80	8	
	T_4	100	20	12	Survival continued
	T_5	100	50	20	
	T_6	100	30	20	
OE-4	T_1	100	50	12	Survival continued
	T_2	100	30	14	
	T_3	100	70	8	
	T_4	100	30	16	
	T_5	100	30	20	Survival continued
	T_6	100	20	20	
OE-9	T_1	100	40	10	Survival continued
	T_2	100	30	12	
	T_3	90	10	8	
	T_4	100	20	14	
	T_5	100	70	20	
	T_6	100	30	20	Survival continued
ME-2	T_1	100	30	10	Survival continued
	T_2	100	20	12	
	T_3	90	60	8	
	T_4	100	50	12	
	T_5	100	20	12	
	T_6	100	50	12	Survival continued
OE-12	T_1	100	30	10	Survival continued
	T_2	0	0	0	
	T_3	0	0	0	
	T_4	100	30	14	
	T_5	100	10	16	
	T_6	100	20	18	

Table 2. Effects of medium-term preservation methods on survival of edible mushrooms

Mushroom Strain	Recovery Date	Per cent recovery of different mushroom strains											
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
S-11	First recovery	100	40	100	100	100	0	60	20	60	60	0	0
	Last recovery	40	20	100	100	20	0	60	20	40	20	0	0
	Survival (months)	12	16	20	20	16	0	8	16	16	12	0	0
	Remarks	-	-	Contd.	Contd.	-	-	-	-	-	-	-	-
U-3	First recovery	80	20	100	100	80	0	60	0	60	60	0	0
	Last recovery	20	20	100	100	20	0	20	0	60	20	0	0
	Survival (months)	12	8	20	20	16	0	12	0	16	16	0	0
	Remarks	-	-	Contd.	Contd.	-	-	-	-	-	-	-	-
NCB-13	First recovery	100	100	100	100	100	0	80	0	100	100	0	0
	Last recovery	20	40	60	80	40	0	40	0	40	40	0	0
	Survival (months)	12	20	20	20	20	0	12	0	16	16	0	0
	Remarks	-	Contd.	Contd.	Contd.	Contd.	-	-	-	-	-	-	-
Pl-20	First recovery	100	100	100	100	100	100	100	100	100	60	20	0
	Last recovery	40	80	80	100	100	60	20	40	80	60	20	0
	Survival (months)	16	20	20	20	20	8	16	12	20	8	8	0
	Remarks	-	Contd.	Contd.	Contd.	Contd.	-	-	-	Contd.	-	-	-
Pl-50	First recovery	60	100	100	100	100	60	100	80	100	100	60	0
	Last recovery	60	40	80	100	60	60	20	20	80	60	60	0
	Survival (months)	8	20	20	20	20	8	20	12	20	12	8	0
	Remarks	-	Contd.	Contd.	Contd.	Contd.	-	Contd.	-	Contd.	-	-	-
Pl-40	First recovery	100	100	100	100	100	100	100	100	100	100	0	0
	Last recovery	20	40	100	100	100	20	20	40	60	20	0	0
	Survival (months)	16	20	20	20	20	16	16	12	20	16	0	0
	Remarks	-	Contd.	Contd.	Contd.	Contd.	-	-	-	Contd.	-	-	-
Pl-10A	First recovery	100	100	100	100	100	0	100	80	100	100	0	20
	Last recovery	20	60	80	100	100	0	20	20	80	80	0	20
	Survival (months)	16	20	20	20	20	0	20	12	20	12	0	0
	Remarks	-	Contd.	Contd.	Contd.	Contd.	-	Contd.	-	Contd.	-	-	-
OE-4	First recovery	60	100	100	100	100	100	40	0	100	100	0	0
	Last recovery	40	40	100	80	100	80	40	0	40	40	0	0
	Survival (months)	12	20	20	20	20	8	8	0	20	16	0	0
	Remarks	-	Contd.	Contd.	Contd.	Contd.	-	-	-	Contd.	-	-	-
OE-9	First recovery	100	60	100	100	100	100	100	100	100	0	0	0
	Last recovery	40	10	40	20	80	80	20	40	60	0	0	0
	Survival (months)	12	20	16	20	20	8	16	12	20	0	0	0
	Remarks	-	Contd.	-	Contd.	Contd.	-	-	-	Contd.	-	-	-
ME-2	First recovery	0	100	100	80	80	0	0	100	60	0	0	0
	Last recovery	0	40	40	20	40	0	0	20	40	0	0	0
	Survival (months)	0	12	16	20	20	0	0	12	8	0	0	0
	Remarks	-	-	-	Contd.	Contd.	-	-	-	-	-	-	-
OE-12	First recovery	0	100	100	40	100	0	0	100	100	0	0	0
	Last recovery	0	20	80	40	20	0	0	80	60	0	0	0
	Survival (months)	0	16	20	16	16	0	0	12	12	0	0	0
	Remarks	-	-	Contd.	-	-	-	-	-	-	-	-	-

Table 3. Effects of long-term liquid nitrogen preservation methods on survival of edible mushrooms.

Mushroom Strain	Treatment	Percent recovery		Period of survival	Remarks
		First recovery (%)	Last recovery (%)		
S-11	T_1	80	60	20	Survival continued
	T_2	100	100	20	Survival continued
U-3	T_1	70	60	20	Survival continued
	T_2	90	90	20	Survival continued
MCB-13	T_1	80	70	20	Survival continued
	T_2	90	90	20	Survival continued
P1-20	T_1	100	100	20	Survival continued
	T_2	100	100	20	Survival continued
P1-50	T_1	100	100	20	Survival continued
	T_2	100	100	20	Survival continued
P1-40	T_1	100	100	20	Survival continued
	T_2	100	100	20	Survival continued
OE-4	T_1	90	90	20	Survival continued
	T_2	100	100	20	Survival continued
OE-9	T_1	0	0	—	Survival continued
	T_2	100	100	20	Survival continued
ME-2	T_1	0	0	—	Survival continued
	T_2	60	60	20	Survival continued
OE-12	T_1	0	0	—	Survival continued
	T_2	90	80	20	Survival continued

0 = (No survival)

T_1 = Fungus preserved in glycerol as mycelial disc.

T_2 = Fungus preserved Glycerol multiplied in wheat grain spawn.

which is a known cryoprotectant, (glycerol) have also given protection to exposed soft mycelial culture disc to a great extent but still during slow cooling cell wall could have been damaged due to change of water present inside the cell wall into ice which eventually ruptured the tender cell wall. Although little metabolic activity takes places below –70°C, re-crystalization of ice crystal growth can cause damage during storage. The volume occupied by water increases by 10% when water crystalizes to form ice. This puts the cell under mechanical stress (Grout and Morris, 1987, Franks, 1981). This explains the reason for less recovery of test strains when preserved as mycelial disc under liquid nitrogen.

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Genetic Diversity Analysis in Soybean with Reference to Pod Shattering

AP Agarwal*, SA Patil Anand and PM Sali Math
Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad-580005, Karnataka

The genetic divergence analysis using Mahalanobis D^2 statistics was carried out in 196 soybean germplasm lines based on the 20 quantitative characters. Estimation of contribution of various characters towards the genetic divergence revealed that individual pod weight was the single largest contributing characters followed by the pod shattering, which is the serious problem in soybean. The 196 germplasm lines were grouped in 11 clusters, of which cluster I accommodated more than 50% of the genotypes. On the contrary four solitary clusters were formed. The 12 shattering resistant genotypes which are identified in the present investigation are distributed in five different clusters indicating the diversity between them and can be utilised in future breeding programme.

Key Words: Genetic Diversity, Pod Shattering, Soybean

A clear understanding of the extent of variability prevalent for each character in germplasm, would imply the scope for improving the character through selection. But in hybridization programme where selection of genetically diverse parents is important to get wide array of recombinants, the knowledge of genetic diversity among the entries of germplasm is necessary. In order to assess the diversity in the germplasm, Mahalanobis D^2 analysis was utilized. The technique has been used in assessing the genetic diversity (Arunachalam and Jawahar, 1967) and proved to be the best tool for selecting the diverse parents for hybridization (Bhatt, 1973).

Pod shattering is an undesirable trait in soybean, known to increase under high temperature and low humidity conditions. The *rabi*/summer season provides the best climate for expression of pod shattering and selection the resistant genotypes. Therefore, the present investigation was carried out to identify the pod shattering resistant genotypes in soybean and to assess the genetic diversity between them.

Materials and Methods

The experiment was conducted with 196 soybean germplasm lines in *rabi*/summer 1999 (December sown). The season was characterized by the high temperature and low relative humidity during the crop maturity, which was conducive for the better expression of pod shattering and associated traits. The experiment was laid out in balanced lattice design with two replications in a single row of 3m with spacing of 45 cm and 15 cm inter- and intra-rows. Observations on six plant growth, four yield attributing and 10 pod characters were recorded. The plant growth characters were days to flower initiation, days to flower termination of main stem, day to maturity, plant height an branches/plant, however, degree of indeterminate growth habit was calculated in day as the difference between days to flower initiation and days to flower termination of main stem. The yield attributing traits were cluster/plant, pods/plant, 100-seed weight (g) and seed yield/plant (g). Pod characters were recorded on the five two-seeded and five three-seeded randomly selected pods. The characters were pod length (mm), pod width (mm), pod thickness (mm), ratio of pod thickness with pod width (PT:PW), ratio of pod thickness with pod length (PT:PL) and seeds/pod. The moisture content of pods at physiological maturity (%), individual pod weight (mg) and seed to pod shell ratio was recorded from the composite sample of 25 pods collected at physiological maturity. However, pod shattering (%) was recorded on the seventh day after maturity as the ratio of number of shattered pods to total number of pods/ plant on five randomly selected plants and expression as per cent. Angular transformed values of pod shattering was used for the statistical analysis.

The genetic divergence analysis was done following method suggested by Mahalanobis (1928), however grouping of the genotypes was done as suggested Rao (1960). The per cent contribution of the characters to the genetic diversity was estimated on rank basis of difference value. The Rank 1 is given to the character exhibiting highest mean different and rank 20 to the character possessing the lowest mean difference. Accordingly, the variable that contributes more towards the divergence was identified by ranking of character-wise D^2 value and adding the rank for each character for all the entries. The percentage contribution of each character was calculated by taking 19110 = 100.

* Correspondence and present address: Zonal Agricultural Research Station, IGAU, Kumhrawand Farm, Jagdalpur, Bastar-494005

Results and Discussion

Evaluation of Genotypes

Mean pod shattering during *rabi*/summer was 51.87% with the range of 1.2% 9 CGP-67-A) to 95.6 (EC-51150-B). Twelve shattering resistant (below 7% shattering) genotypes were identified, of which ten *viz.*,-EC-14396, CGP-67-A, G-101, NRC-12A, GA-95-A, Himso-107, VLS-38, CGP-268 an GP-7340-A are yellow seeded and WC-7 and EC-7033-A are black seeded genotypes.

Contribution of Different Characters Towards Genetic Divergence

The analysis for estimating the contribution of various characters towards the expression of genetic divergence (Table 1) indicats that individual pod weight was the single character which contributed most towards genetic divergence. Though the individual pod weight varied between the node of the same plant (Nakamura *et al.*, 1987), its highest contribution certainly indicates that this character is the basic component of genetic divergence in soybean. Pod shattering which is a serious problem in soybean, contributes next to that of the individual pod weight. The contribution of pods/plant and plant height are also considerable, but the contributions of other character were meager. Kumar and Nandarajan (1994) also reported that pods/plant and plant height were secondary important traits responsible for genetic divergence in soybean.

Tabel 1. Per cent contribution of 20 characters towards soybean genetic divergence

Character	Number of times ranked first	per cent contribution
Days to flower initiation	9	0.04
Days to flower termination	48	0.25
Degree of indeterminate growth habit	10	0.05
Days to maturity	33	0.17
Plant height	554	2.89
Branches/plant	0	0.00
Clusters/plant	12	0.06
Pods/plant	1048	5.48
Seeds/pod	0	0.0
Moisture contents of pods at physiological maturity	57	0.29
Individual pod weight	15373	80.44
Seed to pod shell ratio	0	0.0
Pod length	0	0.0
Pod width	0	0.0
Pod thickness	0	0.0
PT:PL	0	0.0
PT:PE	0	0.0
Seed yield/plant	1	0.0
Pod shattering	1964	10.27
Total	19110	100.00

Grouping of Genotypes into Various Cluster

The 196 genotypes were grouped into 11 cluster based on D^2 values (Table 2). Cluster I accommodated for more than 50% genotypes. This indicates that considerable portion of the germplasm was redundant but the intra-cluster distance was moderate, indicating sufficient diversity between them. In contrast to that, four solitary clusters were formed indicating that these genotypes were diverse from others. The 22 black/brown-seeded genotypes got distributed in four cluster. Cluster I accommodated 11, whereas cluster III had seven and cluster IV had three such genotypes. Remaining one block seeded genotypes was the member of cluster V. The four spreading genotypes were grouped in cluster I (BR-3) and cluster IV (WC-7, EC-7033-A and G-56). This indicates existence of sufficient diversity among the black/brown seeded genotypes. The two shattering resistant checks *viz.*, -Bragg and JS-335 fall in cluster I, whereas Himso-1520 was in cluster II. Two highly susceptible checks Monetta and Block soybean were the members of cluster I and V, respectively. However, MACS-58 (moderately resistant) and JS80-21 (moderately susceptible) checks belongs to cluster III. The resistant genotypes which were identified in the present study are distributed in five different clusters. This suggested the sufficient diversity between them and can be utilized in future breeding programmes.

Intra- and Inter-cluster Distances

The average intra- and inter-cluster distances are presented in Table 3. Cluster XI had the highest intra-cluster distance of 81.64 followed by cluster IV (63.79), cluster III (63.15) and cluster I (62.68). the other intra-cluster distances were 54.41, 48.83 and 37.45 for cluster number II, VI and respectively.

The inter-cluster distances ranged from 53.07 (between two single member cluster VIII and IX) to 606.9 (between cluster VI and XI). Cluster V and XI were the next best diverse clusters as the distance between them was 586.9. Cluster I was closely related to cluster VII (103.2) and cluster II (110.6). Cluster X (119.6) was the next proximal cluster followed by cluster III (125.7). Cluster XI was the farthest from cluster I with D^2 value of 383.6. Cluster II was nearest the cluster IV (111.3) and farthest from cluster VI (332.9). Like wise cluster III was nearest with cluster VII (94.75) and maximum diverse with cluster XI (490.8). The inter-cluster distance between cluster IV with other cluster indicated the lowest distance with VIII (102.4) and highest distance with cluster VI

Table 2. Clustering pattern of 196 soybean genotypes

Cluster number	Number of genotypes	Genotypes
I	108	EC-14396, CGP-67-A, G-101, CGP-85-1, EC-4-A, EC-7033, CGP-927-A, EC-109923, G-95-A, CGP-43, RPSP-227, Pusa-16-A, VLS-9, **EC-11093, G-1080, EC118420, EC-118420-A, BR-3*, IC-18736, AGS-28, CG-103, WC-66-A, CGP-43-B, R2-T21-C,** WC-58, EC-51160-B, EC-51160, AGS-56-B-A, AGS-56-B, AGS-56, IC-100956, G-58, AGS-48, IC-39506, EC-10954, CGP-2426-B, CGP-2426-A, K-202, CGP-927-A-A, CGP-2426-B-A, EC—10954-A, G-78-A, G-58-A, AGS-56-B-C, NIC-19836, CGP-927-B, EC-34092, CGP-253-A, Pusa-16, VLS-45-A, G-75, G-124, G-7340-A, T-35, BR-8, G-22, BS-86-75, G-1412, WC-66, IC-28-A, JS-335, G-130-A, ***Monetta*, Bragg,*** g-1080, CGPB-84, PK-1197, AGS-80, G-140, IC-118233, Line-24, G-147-A, SL-238, KB-83, GP-284, CGP-1405, G-1374, EC-14396-A, ADG-41-A, EC-23-A, WC-23, G-101-A, R2-T38, EC-289073, CGP-268, CGP-1042, GL-38-A, EC-16, IC-47657, CGP-188, CGP-188-A, G-124B, EC-118237-A, JS-1, M-558, WC-16-A, G-147, 94-4, EC-63, WC-4, EC-35657, IC-49859, CGP-85-1.
II	35	NRC-12-A, G-158, EC-391515, G-158-A, CGP-2653, CGP-67, G-156-A, GP-7340-A, PK-262-A, JS-2, TAS-90, IC-11645, IC-104877, G-92-A, EC-104817, R2-T31-B, RSC-2, RSC-3, MACS-45-2, CGPB-84-C, CGPB-84-A, G-540, EC-246, HIMCO-1520, G-92, L-449, G-48, IPS-94, G-237, CGP-108, GP-108, GP-7340-A, EC-118237, G-95, EC-141383, WC-4-A.
III	32	G-74, G-55-1, **EC-109545-A, IC-15997, G-1074, GL-38, G-48-A, G-7340, EC-280129,** EC-39026, AGS-38, G-32, AGS-56-B-B, EC-11843, UGM-20, IC-39506-A, G-124-A, NIC-19836-A, Gaurav, khsB-2, Hardee, IC-28, EC-11893, MACS-58, Ankur, CGP-188-B, G-172-A, WC-15, EC-34083, JS80-21, WC-12-A, WC-12.
IV	6	NRC-12C, PK-262, NRC-12-B, Himso-107, PK-1165, ADG-41.
V	2	6g-78, ***Black Soybean****
VI	7	**WC-7*, EC-7033-A, G-156*,** G-494, CGP-924-B, G-130, G-237-A.
VII	1	1-32
VIII	1	PK-472
IX	1	VLS-38
X	1	EC-109545
XI	2	G-26, AVRDC-20.

Bold = Black seeded genotypes, Bold* = Black seeded spreading genotypes, Italic & Bold = Resistance checks, Italic & Bold* = Susceptible checks

Table 3. Average intra- and inter-cluster distances between 11 clusters in soybean

Cluster*	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	**62.68**	110.6	125.7	200.9	214.4	250.7	103.2	285.4	312.1	119.6	383.6
II	10.52	**54.51**	211.0	111.3	305.1	332.9	170.2	192.8	219.5	155.3	290.7
III	11.21	14.52	**63.15**	306.0	111.3	159.6	93.75	393.2	419.6	145.8	490.8
IV	14.17	10.55	17.49	**63.79**	401.2	425.5	260.0	102.4	131.3	233.3	197.1
V	14.64	17.46	10.54	20.03	**37.45**	102.1	160.8	489.8	515.7	216.6	586.6
VI	15.83	18.24	12.63	20.62	10.10	**48.83**	170.8	514.5	537.6	211.5	606.9
VII	10.16	13.04	9.68	16.12	12.68	13.07	**0.00**	348.1	373.4	64.45	441.1
VIII	16.89	13.88	19.82	10.12	22.13	22.68	18.65	**0.00**	53.07	318.1	108.6
IX	17.66	14.81	20.48	11.45	22.71	23.18	19.32	7.28	**0.00**	344.9	97.96
X	10.93	12.46	12.07	15.27	14.71	14.54	8.02	17.83	18.57	**0.00**	406.6
XI	19.58	17.05	22.15	14.03	24.22	24.63	21.00	10.42	9.89	20.16	**81.64**

* Main diagonal (bold) = inter-cluster D^2 value; Upper diagonal = inter-cluster D^2 value; Lower diagonal = inter-cluster D value

(425.4). Cluster V with cluster and cluster VI with cluster VII and closely related. Cluster VII had lowest distance with cluster x (64.45) and maximum with cluster XI 441.1 Cluster X with the farthest from cluster VIII and IX.

According to Arunachalam *et al.*, (1984) the optimum level of genetic divergence between the parents gives the best heterosis and better segregants. However, Shwe *et al.*, (1972) and Sichkar *et al.*, (1988) suggested that hybridization between highly divergent parent generate promising breeding material.

The method suggested by Arunchalam *et al.*, (1984) was used to classify the genetic divergence present in the germplasm using the intra-and inter-cluster distance. The procedure consisted of the estimation of mean and standard deviation of intra- and inter-cluster distance (D value) and classified into four divergence classes (DC1, DC2, DC3, DC4). As in the present study 11 clusters were formed, of which four contained single variety. Obviously intra-cluster D values for these clusters were zero. Thus, there were 55 inter-cluster, but only seven were intra-cluster D value. 62 D values ranged from 6.11 t 24.63 with the mean of 14.76 and standard deviation of 5.00. Thus, four divergence classes are than DC1 = 24.77 to 19.77, DC = 14.76 to 19.76, DC3=9.75 to 14.75 and DC4 = 9.74 to 4.74.

Cluster classification based on these divergence classes are presented in Table 4. It is evident from the table that, of the 55 inter- cluster distances, 13 come under the divergence class 1, 15 and 24 inter cluster distances come under the divergence class 2 and 3respectively. However, only three inter cluster distances represent the divergence classes (DC-2 and DC-3) from the other clusters. However, cluster III and VII represent all the four divergence class distance with other clusters.

Twelve shattering resistant genotypes were distributed in five different clusters, i.e. cluster I, II, IV, VI and IX. These clusters belong to divergence class DC2 and DC3, except the cluster VI from IV and IX comes under DC1. Moreover the genotypes belongs to cluster VI work black seed genotypes.

Individual D^2 values between the genotypes indicated that EC-14396, CGP-67-A, G-0101 were close, but Bragg had optimum diversity with others in cluster I, whereas NRC-12-A and EC-3911515 had optimum distance in cluster II. Intermitting of these genotypes or crossing with Himso-107 (cluster IV) and VLS 38 (Cluster IX) may give better chance for developing shattering resistance genotypes.

Cluster Mean Analysis

The study of clusters mean value of 11 clusters indicated considerable differences for the characters studies (Table 5). After leaving out the solitary clusters, the range of variation for days to flower initiation, seeds/ pod, PT:PL and PT:PW were low among the multi-member clusters. The characters *viz.*- days to flower termination, degree of indeterminate growth habit, 100-seed weight, moisture content of pod at physiological maturity, seed to pod shell ratio, pod length, pod width and pod thickness exhibited moderate variations. The high range of variation was observed for days to maturity, plant height, branches/plant, cluster/plant individual pod weight, seed yield and pod shattering among the different clusters.

The variations observed in cluster mean also point out the degree of variability. In the present investigation, clusters I, II, IX had early flowering initiation genotypes, whereas cluster I, II, IV, VIII, IX and XI had early flowering termination genotypes than the population mean. Cluster I, IV, VIII, IX and XI had lower degree for indeterminate growth habit. The genotypes included in clusters I, IX and XI recorded early maturity. Above

Table 4. Classification of inter-cluster distances into four divergence classes*

Divergence class	Divergence cluster	
	From	To
DC1	III	VIII, IX, XI
	IV	V, VI
	V	VIII, IX, XI
	VI	VIII, IX, XI
	VII	XI
	X	XI
DC2	I	VI, VIII, IX, X
	II	V, VI, IX, XI
	III	IV
	IV	VII, X
	VII	VIII, IX
	VIII	X
	IX	X
DC3	I	II, III, IV, V, VII, X
	II	III, IV, VII, VIII, X
	III	V, VI, X
	IV	VIII, IX, XI
	V	VI, VII, X
	VI	VII, X
	VIII	XI
	IX	XI
DC4	III	VII
	VII	X
	VIII	IX

* According to Arunachalam *et al.*, (1984)

Table 5. Cluster mean for 20 quantitative characters in soybean

Character	Cluster number											GM
	I	II	III	IV	V	VI	VII	VIII	IX*	X*	XI	
DFI	44.4	44.1	46.3	45.1	47.0	46.2	55.5	47.0	41.5	62.5	45.5	44.93
DFT	58.3	59.1	61.8	56.7	64.8	63.4	75.0	57.0	49.5	81.0	57.3	59.30
DID	13.8	15.0	15.5	11.6	17.8	17.2	19.5	10.0	8.0	18.5	11.8	14.38
DM	94.7	97.0	97.4	97.3	98.3	102.6	103.0	99.0	94.0	112.5	96.0	96.11
PH	34.2	34.1	43.4	27.9	62.3	60.2	72.6	27.6	47.7	56.5	40.7	37.10
BP	2.9	3.3	3.5	2.9	4.4	4.2	5.0	2.1	3.6	6.0	2.6	3.13
CP	17.7	19.1	21.1	16.5	25.3	32.5	35.3	12.4	9.7	46.8	19.6	19.25
PP	42.5	47.9	50.5	41.5	67.3	78.8	101.3	29.0	22.1	141.0	44.4	46.96
SP	2.4	2.5	2.3	2.3	2.3	2.2	2.8	2.2	2.5	2.6	2.5	2.42
SW	11.0	12.3	9.0	13.4	10.3	11.7	15.0	13.9	19.4	5.6	14.4	11.10
MCP	57.0	57.3	56.7	56.4	59.5	56.6	57.3	39.6	59.7	52.7	45.2	56.8
IPW	423.5	527.4	316.4	569.8	315.0	452.0	370.0	705.5	730.0	413.5	803.3	435.5
S:PS	1.6	1.6	1.6	1.7	1.6	1.7	1.9	0.5	1.9	1.9	0.7	1.61
PL	4.17	42.9	39.8	42.6	38.8	40.4	42.0	41.6	52.8	41.5	47.1	41.6
PW	9.3	9.6	8.8	10.0	8.0	8.9	8.9	9.3	10.4	8.9	9.9	9.27
PT	5.4	5.5	4.6	5.6	4.8	4.7	4.9	4.7	5.2	4.9	5.6	5.23
PT:PL	0.130	0.133	0.127	0.117	0.127	0.120	0.102	0.106	0.098	0.118	0.119	0.126
PT:PW	0.582	0.577	0.570	0.526	0.545	0.550	0.455	0.503	0.500	0.551	0.569	0.566
SY	4.3	5.0	4.0	4.5	3.9	5.7	6.5	2.9	5.5	7.9	2.7	4.47
PSh	52.0	48.1	59.5	36.7	86.1	43.1	50.5	48.9	4.0	46.2	55.3	51.87

* Solitary clusters

average plant height was found in most of the clusters, but cluster III and IX had moderate plant height with increased branches/plant. So the cluster I and IV had desirable means for plant growth characters.

For the yield attributing trait cluster III, V and VI had higher means for clusters/plant and pods/plant. 100-seed weight was more in cluster, II, IV, VI, XI and solitary clusters except cluster X. These results imply that cluster VI possessed the desirable mean for yield attributing traits, part from solitary clusters VII and X.

The mean of clusters of important pod characters indicates that individual pod weight was higher in clusters II, IV, VI, VII, IX and XI, whereas clusters II, IV, VII and IX and XI possessed the higher pod length. The higher seed to pod shell ration was observed in clusters IV, VI, VII, IX and X. Although the seven clusters had lower pod shattering than the population mean, a solitary cluster IX had the desirable means for important pod characters. In addition to this cluster IV had mean in desirable direction for plant yield and pod characters. So the genotypes included in this cluster can have more utility value for overall improvement.

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Comparative Analysis of Variability in Two Ecotypes of Indian Rapeseed (*Brassica campestris* L. var. *Yellow* and *Brown Sarson*)

SK Yadav, AK Shukla, JS Chauhan, AK Singh and PR Kumar
National Research Centre on Rapeseed-Mustard, Sewar, Bharatpur-321303, Rajasthan

One hundred and fifty germplasm accessions of two ecotypes of Indian rape (*Brassica campestris* L.) namely var. brown sarson (90 accessions) and var. yellow sarson (60 accessions) were characterized for 10 agro-morphological traits. Wide range in means were observed in the two ecotypes. The accessions of brown sarson matured one week earlier and also had slightly less height than that of yellow sarson. Mean secondary branches/plant, seeds/siliqua and 1000 seed weight were higher in yellow sarson ecotype than that of brown sarson. Irrespective of the group, oil content (CV 2.7-2.9%) and plant height (CV3.7-4.1%) had the lowest variation whereas, primary branches/plant (CV 27.0-35.9) and secondary branches/plant (CV 38.4-49.1%) the highest. The useful accessions for different agro-morphological traits were identified from both the ecotypes. The pattern of grouping is presented and 10 accessions with high oil content (≥45%) are also listed and characterized.

Key Words: ***Brassica campestris* var. *yellow sarson* and *brown sarson*, Germplasm, Oil Content**

Rapeseed (*Brasica campestris* L.) is an important oilseed crop among the major edible oilseeds. There are there ecotypes of *B. campestris*, namely var. toria, var. yellow sarson and var. brown sarson. Although var. brown and yellow sarson are cultivated on a limited scale in certain pockets of Jammu and Kashmir, Assam, Bihar, West Bengal, Himachal Pradesh and Haryana. Owing to earliness and high oil content they have good scope of spreading if high yielding varieties are available. Large number of accessions of brown and yellow sarson were available at the Project Coordinating Unit (Kumar, 1987) which were later shifted to National Research Centre on Rapeseed-Mustard, Bharatpur. With a view to find out their potential, 150 germplasm accessions were characterized for 10 agro-morphological traits in the present investigations.

Materials and Methods

One hundred fifty accessions of *Brassica campestris* L. var. yellow sarson (60) and brown sarson (90) were grown in augmented design during *rabi* season of 1995-96 at National Research Centre on Rapaseed-Mustard, Bharatpur. Each accession was sown in two rows of 5 m with the spacing of 15 cm and 30 cm plant-to-plant and row-to-row, respectively. Recommended dosages of fertilizers (N and P @ 80:17 kg/ha) were applied. Half of the nitrogen (40 kg/ha) and full dose of phosphorus were applied at the time of sowing while remaining half of the nitrogen (40 kg) was top dressed just after first irrigation (40 days after sowing). Suitable agronomic practices and plant protection measures were followed as and when needed.

Observations were recorded on five competitive randomly chosen plants. The characters studied were days to maturity, plant height (cm), primary branches/plant, secondary branches/plant, main shoot length (cm), siliquae on main shoot, siliqua length (cm), seeds/siliqua, 1000-seed weight (g) and oil content (%).

An electronic seed counter ("L" System Old Mill Co. USA) was used for counting 1000-seeds and the oil content (%) was analysed by Nuclear Magnetic Resonance (Oxford 5000). Range, mean and coefficient of variations (CV) computed separately for brown and yellow sarson accessions. The accessions were grouped following appropriate class intervals.

Results and Discussion

To adopt in the multiple cropping production system, the varieties of appropriate plant height such as short height and early maturity are highly desirable for mixed cropping. Indian rape accessions are of relatively early- to mid-early duration as compared to other oleiferous *Brassica* crops. Both the ecotypes, *viz.*- brown sarson and yellow sarson had similar extent of variability for plant height (Table 1). Plant height ranged from 138 to 218 and 115 to 193 cm, in yellow sarson and brown sarson, respectively. The mean plant height of yellow sarson accessions was slightly more than that of brown sarson. Thirty two accessions of yellow sarson had plant height of ≤ 155 cm whereas in the brown sarson, majority of the accessions (63.3%) fell between 156-175 cm (Fig. 1a). In the present investigation, 56.7% of the brown sarson and 5% of yellow sarson accessions matured during less than 120 days. In general, yellow sarson accessions matured later than those of brown

sarson (Fig. 1b). Although, yellow sarson exhibited wider range of maturity than brown sarson, the overall variability was low (CV 3.7-4.1%). Accessions having early duration (≤ 120 days) and short plant height (137-160 cm) have also been identified (Table 2).

Main shoot length and siliquae on main shoot have been reported to have high heritability hence very useful in the selection of high yielding genotypes. Range and mean shoot length of the yellow sarson collection were slightly more than those of brown sarson (Table 1). The

Table 1. Range, mean and coefficient of variation (CV) for various economically important characters of yellow and brown sarson

Character	Ecotypes	Range	Mean ± S.Em.	CV(%)
Maturity (Days)	Yellow sarson	117 (YSPG 843) -140 (YSC 21)	129.9 ± 0.7	4.1
	Brown sarson	116 (BSC 45) - 132 (BSC 152)	121.3 ± 0.5	3.7
Plant height (cm)	Yellow sarson	138 (PYS 386) - 218 (YSC 18)	174.4 ± 1.5	8.4
	Brown sarson	11S (BSC 51) - 193 (BSC 64)	164.1 ± 1.5	8.5
Primary branches/plant	Yellow sarson	4.0 (YSC 28) - 18.0 (SSK92-16)	8.0 ± 0.4	35.9
	Brown sarson	3.8 (BSC 68) 16.2 (BSC 46)	8.3 ± 0.2	27.0
Secondary branches/plant	Yellow sarson	2.0 (PYS 286) 14.6 (YSC 10)	8.0 ± 0.4	38.4
	Brown sarson	2.5 (BSC 80) 16.6 (BSC 46)	5.8 ± 0.3	49.1
Main shoot length (cm)	Yellow sarson	55.8 (PYS 386) 89.6 (YSC 31)	72.8 ± 1.1	12.0
	Brown sarson	37.4 (BSC 71) 82.2 (BSC 97)	67.3 ± .09	12.5
Siliquae on main shoot	Yellow sarson	32.0 (YSC 27) 51.2 (YSC 20)	41.4 ± 0.6	11.7
	Brown sarson	19.0 (BSC 46) 83.6 (BSC 98)	41.4 ± 0.8	18.9
Siliqua length (cm)	Yellow sarson	3.9 (YSPG 187) 6.4 (YSC 36)	5.4 ± 0.1	9.2
	Brown sarson	4.2 (BSC 56) 6.7 (BSC 147)	5.4 ± 0.1	10.6
Seeds/siliqua weight (g)	Yellow sarson	15.6 (YSC 22) 44.6 (YSPG 387)	24.0 ± 0.8	25.0
	Brown sarson	12.2 (BSC 81) 23.4 (BSC 73)	19.1 ± 0.2	10.2
1000-seed weight (g)	Yellow sarson	4.0 (YSC 35) 6.9 (SSK 6)	5.3 ± 0.1	13.2
	Brown sarson	3.1 (BSC 73) 5.9 (BSC 139)	4.7 ± 0.1	11.5
Oil content (%)	Yellow sarson	41.0 (YSC 54) 47.0 (YSC 32)	44.2 ± 0.2	2.9
	Brown sarson	40.5 (BSC 133) 45.9 (BSC 63)	43.5 ± 0.1	2.7

Table 2. Useful donors for various characters of yellow and brown sarson

Character	Crop	Promising accessions
Maturity (days)	Brown sarson	BSC 45 (116), BSC 48 (116), BSC 66 (116) BSC 92 (116) BSC 53 (117).
	Yellow sarson	YSPG 843 (117), SSK 13 (118), YSC 9 (120), YSPG 847 (121), YSC 11 (122).
Plant height (cm)	Brown sarson	BSE 51 (115.2), BSC 46 (116-6), BSC 148 (141.4), BSC 69 (144.4), BSC 121 (149.4).
	Yellow sarson	PY 386 (137.8), YS 13 (145.2), YSC 20 (158.0), YSPG 287 (159.0), YSCN 7 (159.2).
Primary branches/plant	Yellow sarson	YSCN 2 (18.0), SSK 92-16 (18.0), YSC 10 (14.6), YSC21 (1 3.6),YSC 6 (12.6).
	Brown sarson	BSC 46 (16.2), BSC 47 (14.0), BSC 140 (13.6), BSC 129 (11.8), BSC 114 (10.4).
Secondary branches/plant	Yellow sarson	YSC 10 (14.6), YSC 34 (10.8), YSC 53 (10.7), YSC 50 (10.6), YSC 27 (10.4).
	Brown sarson	BSC 46 (16.6), BSC 123 (14.7), BSC 129 (11.5), BSC 150 (9.8), BSC 124 (9.7).
Main shoot length (cm)	Yellow sarson	YSC 31. (89.6), YSC 32 (85.6), YSC 26 (84.4), YSC 10 (84.0), YSC 36 (83.2).
	Brown sarson	BSC 97 (82.2), BSC 78 (81.5), BSC 76 (81.2), RSC 80 (81.2), BSC 90 (80.4).
Siliquae on main shoot	Yellow sarson	YSC 20 (51.2), YSCN 2 (50.2), YSPG 387 (50.2), YSC 10 (50.0), YSC 442 (50.0).
	Brown sarson	BSC 98 (83.6), BSC 85 (79.8), BSC 87 (79.4), BSC 84 (74.2), BSC 99 (71.0).
Siliqua length (cm)	Yellow sarson	YSC 36 (6.4), YSC 43 (6.2), YSC 44 (6.2) YSC 53 (6.2), YSC 39 (6.1).
	Brown sarson	Bse 147 (6.7), BSC 121 (6.2), BSC 122 (6.2), BSC 136 (6.2), BSC139 (6.2).
Seeds/siliqua	Yellow sarson	YSPG 387 (44.6), PYS 286 (43.6), PYS 386 (40.0), YSPG 186 (37.0), YSPG 847 (33.6).
	Brown sarson	BSC 73 (23.2), BSC 80 (23.2), BSC is (21.6), BSC66 (21.2), BSC 53 (21.0).
1000-seed weight (g)	Yellow sarson	SSK 6 (6.9), YSC 9 (6.8), YSC 50 (6.0), YSC 51 (6.0), YSC-27 (6.0).
	Brown sarson	BSC 139 (5.9), BSC 147 (5.8), BSC 50 (5.8), BSC 140 (5.7), BSC 145 (5.6).
Oil content (%)	Yellow sarson	YSC 32 (47.0), YSC 10 (46.3), YSC 31 (46.2), YSPG 8143 (45.8), YSC 21 (45.7).
	Brown sarson	BSC 63 (45.9), BSC 92 (45.8), BSC 78 (45.4), BSC 155 (45.2), BSC 61 (45.1).

Mean values are in parentheses

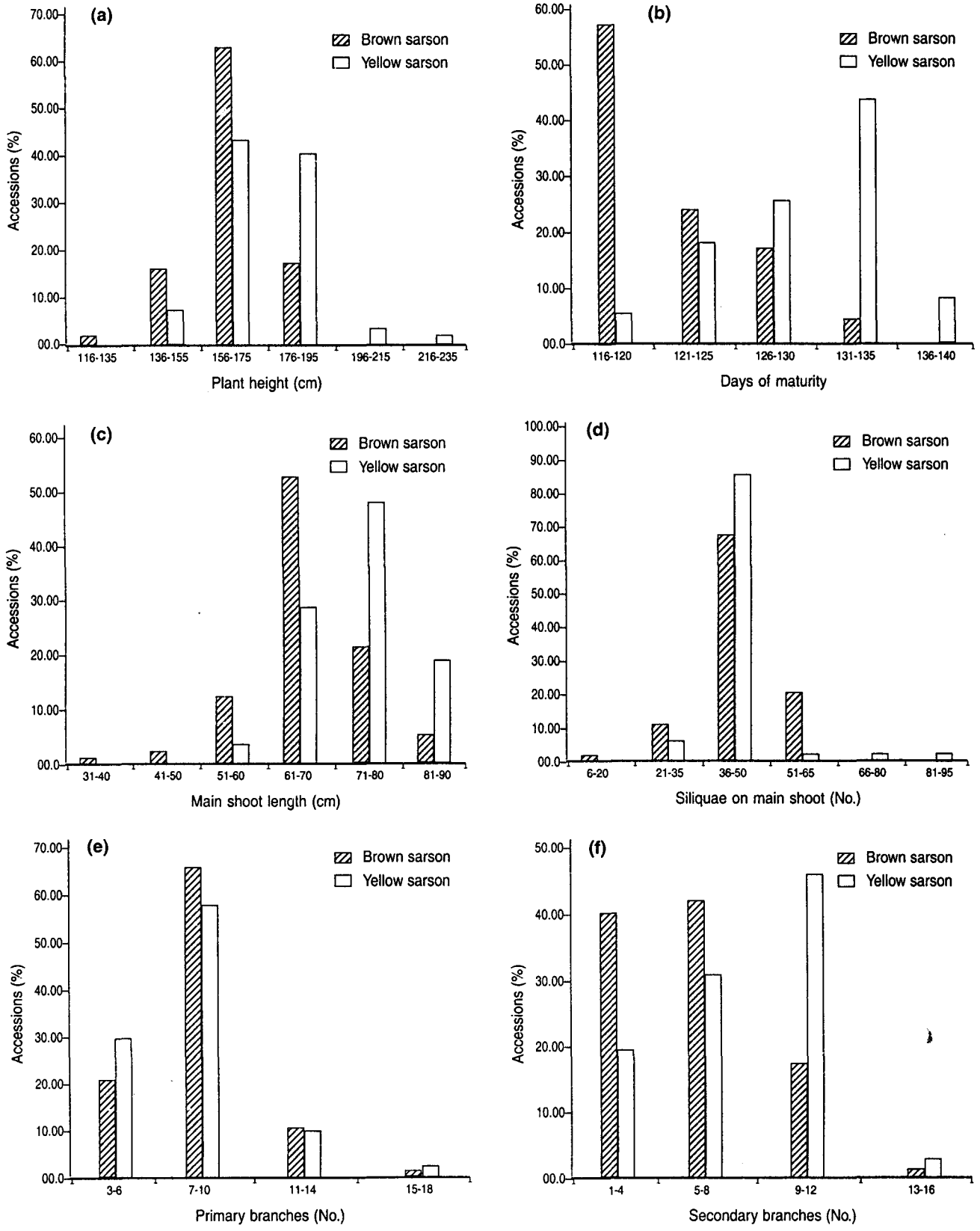

Fig. 1a-f. Classification of brown sarson and yellow sarson germplasm accessions based on (a) plant height, (b) days to maturity (c) main shoot length, (d) siliquae on main shoot, (e) primary branches (f) secondary branches

difference between variability in the two collections was not significant. Siliquae on main shoot varied more in brown sarson collections (CV 18.9%) than in yellow sarson (CV 11.7%). Kumar and Yadava (1978) also reported substantial variability in Indian colza. However, there was no difference for siliquae on main shoot (Table 1). The grouping of accessions based on main shoot length (Fig. 1c) revealed that 44.4% and 43.3% of the accessions had ≥ 70 cm long main shoot in brown sarson and yellow sarson collections, respectively. In general, most of the accessions (86.7%) of the yellow sarson collection had siliquae on main shoot ranging from 26-35 (Fig. 1d) whereas siliquae density (siliquae/cm) appeared more in brown sarson collection where (64.4%) accessions grouped in the range of 36–50 siliquae. Further 17 accessions (18.9%) had more than 50 siliquae on the main shoot (Fig. 1d). Accessions with long main shoot and high siliquae on main shoot are listed in Table 2.

In contrast to all the other characters studies, branches/plant and secondary branches/plant varied significantly within different accessions in both the groups (Table 1). Of the two, primary branches/plant had higher variation in yellow sarson collection whereas it was secondary branches/plant in brown sarson which showed higher variability (Table1). Similarly, Yadava *et al.*, (1985) reported high variability for primary branches/plant in brown sarson. The mean in general, was similar for both the collections for primary branches/plant whereas in brown sarson collection secondary branches/plant was less as compared to that of yellow sarson The grouping of the accessions on the basis of primary branches/plant and secondary branches/plant has been depicted in Figs. 1e and 1f. Only two accessions, one each from brown sarson (BSC 46) and yellow sarson (SSK 92-16) had very high number of primary branches/plant (≥14) and three accessions had high number of secondary branches (13-16), *viz.*- BSC 46, BSC 123 and YSC10 (Table 2).

Siliqua length ranged from 3.9 (YSPG 187) to 6.4 (YSC 36) and 4.2 (BSC 56) to 6.7 cm (BSC 147), respectively in yellow sarson and brown sarson ecotypes. Mean siliqua length in both ecotypes was similar, though brown sarson collection had slightly higher variability (Table 1). Most of the accessions of yellow sarson (41, 68.3%) had siliqua length in the range of 5.1-5.5 cm whereas 65.6% of the brown sarson accessions (59) were grouped in the range of 5.5-6.0cm (Fig 1g). Five accessions of each ecotype showed siliqua more than 6 cm (Table 2).

The yellow sarson collection exhibited higher range, mean and coefficient of variation for seeds/siliqua as compared to that of brown sarson (Table 1). Most of the brown sarson accessions (72) had less than or equal to 20 seeds/siliqua while 12.2% (11) showed 21-25 seed/siliqua whereas 7 accessions of yellow sarson had more than 25 seeds (Fig. 1h).

Bold-seeded varieties are the preferred ones. This character had moderate variability (CV 11.5-13.2%). Yellow sarson collection exhibited slightly higher mean

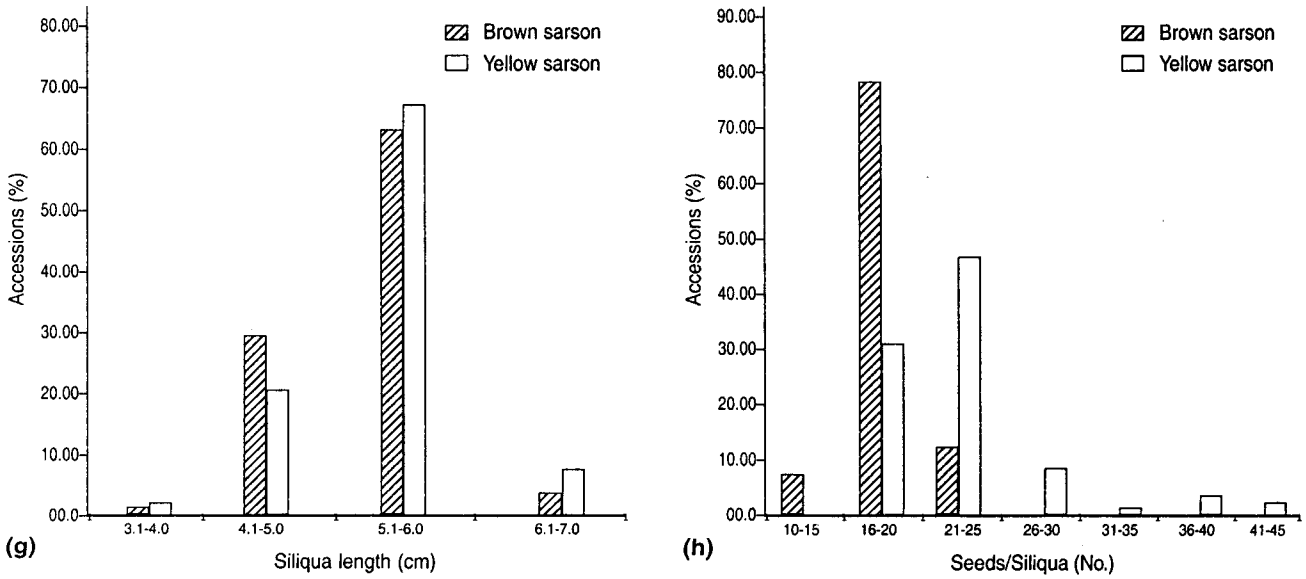

Fig. 1g-h. Classification of brown sarson and yellow sarson germplasm accessions based on (g) siliqua length, (h) seeds/siliqua

(Table 1). Five accessions of yellow sarson exhibited high seed weight of 6 g (Table 2) whereas in brown sarson, the highest seed weight was 5.9g (BSC 139). Sixty one accessions (67.8%) of brown sarson showed 1000-seed weight ranging from 4.1-5.0 g while 61.6% (37) of the yellow sarson accessions showed a range of 5.1-6.0g (Fig. 1i).

Oil yield determines the commercial success of the variety. Among all the characters, irrespective of the ecotypes, oil content varied the least (CV 2.7-2.9%). The oil content varied from 41-47 and 40.5-45.9% respectively in the yellow sarson and brown sarson ecotypes. YSC 32 of yellow sarson and BSC 63 of brown sarson had the highest oil content (Table 1). There was marginal increase in mean oil content of yellow sarson accessions. The agro-morpholigical characters of 10 top high oil yielding accessions are presented (Table 3). Fifty two accessions (57.7%) among the brown sarson

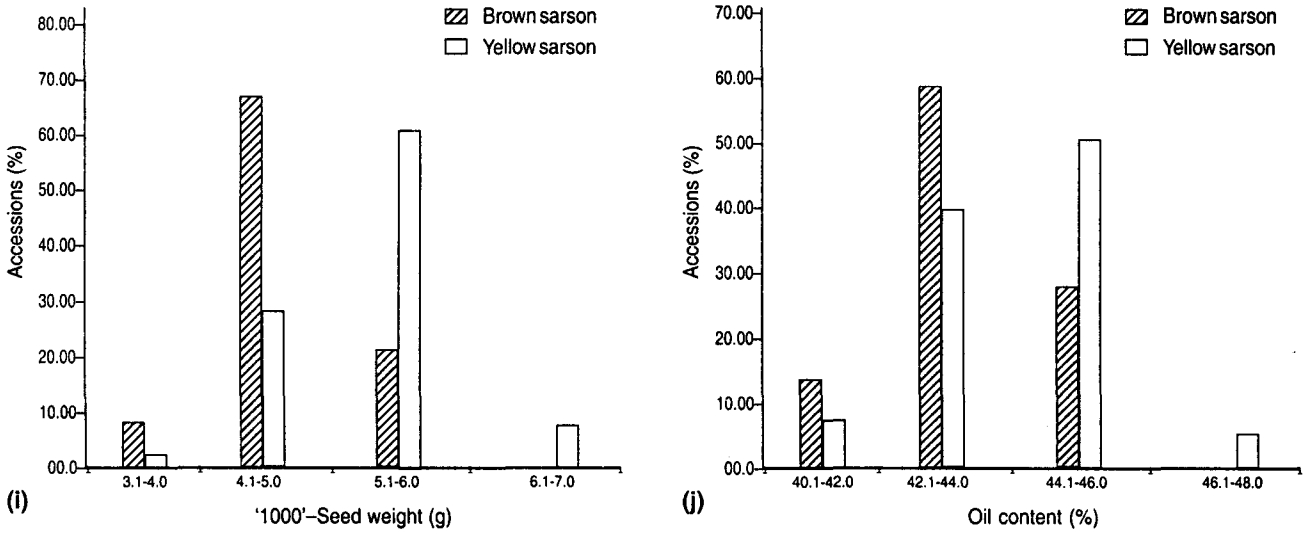

Fig. 1i-j Classification of brown sarson and yellow sarson germplasm accessions based on (g) siliqua length, (h) seeds/siliqua

Table 3. Characters of yellow and brown sarson accessions having high oil content

Genotype	Oil (%)	Maturity (days)	Plant (cm)	Primary plant	Secondary plant	Siliquae on main shoot	Siliqua (cm)	Seeds/ siliqua	1000-seed seed
YSC-32	46.9	129	190	6	4.2	44	5.1	20	5.6
YSC-10	46.3	119	182	12	14.6	50	5.0	18	5.6
YSC-31	46.2	130	200	10	3.0	49	4.7	22	5.5
YSPG-8143	45.8	117	171	10	8.5	40	4.9	27	4.1
YSC-21	45.7	140	163	6	3.6	37	5.1	20	5.6
BSC-63	45.9	118	154	12	5.0	31	5.7	21	4.4
BSC-92	45.8	116	176	5	3.8	39	4.8	20	3.8
BSC-78	45.4	130	171	8	3.5	52	4.8	20	4.3
BSC-155	45.2	122	154	8	6.0	34	5.9	20	4.4
BSC-61	45.1	121	156	7	3.8	40	5.1	19	48

collection had oil content in the range of 43-44% whereas 25 had a range of 45-45.9%. Three accessions of yellow sarson *viz.* YSC 32, YSC 10 and YSC 31 had more than 46% oil content (Fig. 1j).

Several useful accessions possessing desirable agro-morphological characters and oil content were identified in the present collections which could serve as an important genepool for the hybridization programme to improve yield and oil content *Brassica* species.

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Exploration and Collection of Citrus Germplasm from NEH Region (Meghalaya) of India

IP Singh, Shyam Singh, R Srivastava* and K Singh*

National Research Centre for Citrus, P.B. 464, Shankarnagar P.O., Amravati Road, Nagpur-440010

**NBPGR, Regional Station, Umiam, Meghalaya*

The North-Eastern hills region of India is considered as one of the natural home of many citrus species. Availability of favourable climatic conditions and easy hybridization among different species and genera has resulted in numerous wild/semi-wild forms in North-eastern states of India. Surveys were conducted in Khasi, Jaintia and Garo hills of Meghalaya to collect citrus germplasm from these regions including those from citrus gene sanctuary. A total of 79 accessions belonging to 15 *Citrus* species and 3 new types (probably hybrids) were collected. Five endangered citrus species, namely, *C. indica, C. latipes, C. macroptera, C. assamensis,* and *C. megaloxycarpa* were also collected. The collected materials were regenerated at NRCC, Nagpur and NBPGR, Regional Station, Umiam, Meghalaya and maintained under good conditions. The materials will be planted in field gene bank for evaluation and utilization.

Key Words: Citrus, Exploration, Germplasm, Meghalaya

North-Eastern hill (NEH) region , North-Western (NW) region particularly the foothills of Himalayas and Southern peninsular region of India, are considered the major centres of citrus diversity. A large number of citrus species/progenitors of commercial citrus fruits are believed to have originated in India (Bhattacharya and Dutta, 1951, 1956; Dutta, 1958; Singh, 1967; Singh and Singh 1967, 1968). Many of these species are wild. Hore *et al.*, (1997) reported many citrus species in NEH region. The need to collect and conserve the genetic resources is far greater in present time than ever before because the number of cultivated types has narrowed to only the best available ones with regards to productivity and quality. There is also a need to protect the collected germplasm since many of the field genebanks are under deteriorated condition due to several factors.

Until recently, desired genotypes and landraces, mostly of commercial value, were collected and maintained by individual farmers. National Research Centre for Citrus, Nagpur, which is the main centre for augmentation of Citrus germplasm, has conducted surveys in NEH region and collected citrus genetic resources.

Materials and Methods

An exploration was undertaken in collaboration with NBPGR, Regional Station, Umiam, Meghalaya and total 79 collections were made from Shillong, Cherapunji, Shella area of East Khasi Hills, Jowai, Muktapur and Dawki area of Jaintia Hills, Ribhoi area of Khasi Hills, Kamrup area of Assam, Tura, Rongram, Chandigre and Darichickgre area of West Garo hills and Dalu, Siju and Baghmara area of South Garo Hills and Ranikor area of West Khasi Hills. The habitat covered rocky, undulating mountainous slopes, disturbed, eroded slopes and backyards. Collection sites encompassed a wide altitudinal range *viz.*, 250 m to 1700 m. The soils ranged from shallow to deep soils. The rainfall pattern also varied from 2000-4000 mm per year. Budsticks and mature fruits were collected. Fruits were picked randomly and used for analysis of their physico-chemical properties. Collected seeds were dried in shade and sown in primary nursery for germination.

Table 1. Accessions of *Citrus* spp. collected during the exploration in Meghalaya

No. of accessions	Species	Common name	Occurrence
31	*C. grandis*	Pummelo	Home garden
6	*C. jambhiri*	Sohmyndong	Home garden
1	*C. macroptera*	Satkara	Semi wild
2	*C. reticulata*	Khasi mandarin	Cultivated
2	*C. latipes*	Soh Shyrkhoit	Home garden
1	*C. megaloxycarpa*	Sour Pummelo	Home garden
7	*C. limon*	Jaintia lemon	Cultivated
8	*C. medica*	Citron	Home garden
3	*C. assamensis*	Ada jamir	Home garden
6	*C. pseudolimon*	Galgal/Chinara	Home garden
3	*Probable Hybrid*	Chambil	Home garden
4	*C. indica*	Memon Narang	Wild/semi wild
2	*C. limmetta*	Sharbati lime	Cultivated
1	*C. madurencis*	Calamondin	Home garden
1	*C. aurantium*	Karun jamir	Home garden
1	*C. limettioides*	Sweet lime	Home garden

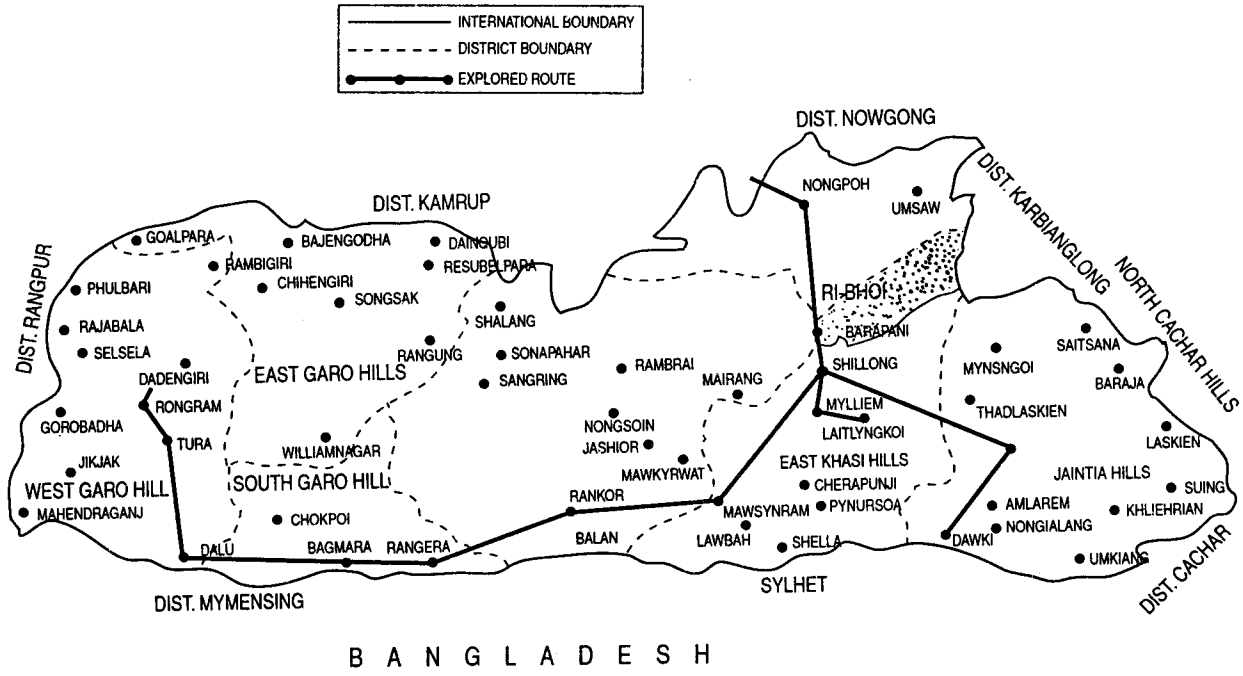

Fig. 1. Route followed during exploration

Results and Discussion

A wide range of variability was observed in collected material. A distinct variation was found in fruit weight, number of segment, fruit length, fruit width, number of seed/fruit and rind thickness (Table 2). Fruit colour was varied from yellow to reddish-deep yellow and greenish yellow. Bearing habit, fruit shape and thorny habit showed distinct variability.

Cherapunji and Shella area of East Khasi Hills and Jowai area of Jaintia Hills were the potential areas for Khasi mandarin and other citrus species. However, due to excess rain and deforestation, these areas are not considered potential area now for citrus except Dawki and Muktapur area in Jaintia Hills. Different types of citrus were collected from Muktapur area including 3 endangered *Citrus* species (*C. latipes, C. assamensis, C. megaloxycarpa*). *C. latipes* grows only at higher altitude above 900 m. around Shillong area whereas Satkara (*C. macroptera)* grows only in lower altitude less than 500 m in semi-wild form. It has good market value in comparison to Khasi mandarin. Area bordering to Bangladesh have greater variability for Pummelo, Satkara and Citron. Ribhoi area of Khasi hills is good for Khasi mandarin, Pummelo, Lemon and Citron, different types were collected from this area. Maximum variability was observed in Pummelo and Citron. In Kamrup area of Assam, different types of Assam lemon and Pummelo occurred.

The Garo Hills of Meghalaya is one of the area that harbours highest citrus genetic diversity. The Citrus gene sanctuary is well protected and plants are less susceptible to infection. The Nokrek Biosphere reserve have maximum genetic diversity of citrus. From here 3 types of Memon Narang (*C. indica*) were collected. Variability was observed in fruit size, colour and shape. Three new types, probably hybrids were also collected from this area. Siju and Baghmara area of West Garo Hills are rich in Pummelo, Khasi mandarin, Sharbati lime, Citron and Chinara germplasm.

Conclusions

The threat of genetic erosion of citrus diversity is not there but farmers preferences play a vital role in species survival. Due to this reason the genetic diversity in sour pummelo, white fleshed pummelo, *C. latipes* are fast eroding. Citrus plants in citrus gene sanctuary are well protected in healthy condition than plants found outside. Satkara (*C. macroptera*) that grows in semi-wild form in Shella area of Cherrapunji, now faces problem due

Table 2. Variation in physico-chemical characteristics of fruits in collected germplasm

S. No.	Collector No.	Species/Landraces	Fruit wt (g)	Fruit length (mm)	Fruit breadth (mm)	Peel thickness (mm)	No. of segment	Seeds/ fruit	TSS	Acidity (%)
1.	NRCC-241	Pummelo (*C. grandis*)	445.38	87.45	99.75	8.95	16.84	71.34	9.56	1.36
2.	NRCC-242	Sohmyndong (*C. jambhiri*)	75.00	52.66	48.60	2.74	9.00	12.60	6.70	4.35
3.	NRCC-243	Pummelo (*C. grandis*)	567.45	98.56	112.54	10.43	15.80	45.77	9.56	1.45
4.	NRCC-244	Sohmyndong (*C. jambhiri*)	69.35	50.42	49.33	2.56	8.5	16.00	7.00	4.80
5.	NRCC-245	Satkara (*C. macroptera*)	192.5	71.77	81.28	4.2		11.2	8.40	8.07
6.	NRCC-246	Khasi mandarin (*C. reticulata*)	–	–	–	–	–	–	–	–
7.	NRCC-247	Pummelo (*C. grandis*)	567.45	82.34	97.65	8.45	15.43	82.34	9.78	1.56
8.	NRCC-248	Pummelo (*C. grandis*)	487.46	79.569	90.35	10.56	16.45	69.86	10.00	1.34
9.	NRCC-249	Pummelo (*C. grandis*)	623.76	86.53	102.25	11.54	15.00	65.34	11.00	2.10
10.	NRCC-250	Soh Shyrkhoit (*C. latipes*)	342.45	81.24	90.34	12.45	12.00	29.67	9.00	6.69
11.	NRCC-251	Pummelo (*C. grandis*)	398.00	95.00	111.00	14.00	16.00	120.00	9.67	1.38
12.	NRCC-252	Soh Shyrkhoit (*C. latipes*)	424.00	86.50	105.60	14.80	11.00	61.00	7.6	8.6
13.	NRCC-253	Sour Pummelo (*C. megaloxycarpa*)	378.00	91.60	98.30	13.00	15.00	54.00	8.6	6.8
14.	NRCC-254	Jaintia lemon (*C. limon*)	120.00	80.30	58.75	5.00	11.00	58.00	6.7	5.6
15.	NRCC-255	Citron (*C. medica*)	106.24	86.75	65.45	4.60	13.00	36.78	7.00	6.95
16.	NRCC-256	Citron (*C. medica*)	54.66	72.33	41.83	3.00	14.00	56.66	6.50	7.80
17.	NRCC-257	Citron (*C. medica*)	79.56	68.67	64.23	4.85	14.00	35.54	7.00	6.34
18.	NRCC-258	Pummelo (*C. grandis*)	628.00	101.30	123.70	11.20	20.00	89.00	9.4	1.70
19.	NRCC-259	Pummelo (*C. grandis*)	478.00	110.40	111.70	14.40	15.00	161.00	10.2	2.0
20.	NRCC-260	Pummelo (*C. grandis*)	596.00	97.00	104.70	6.70	16.00	73.00	10.20	1.80
21.	NRCC-261	Pummelo (*C. grandis*)	882.00	122.80	133.00	12.00	19.00	162.00	10.00	2.20
22.	NRCC-262	Nimbu (*C. jambhiri*)	22.40	38.54	35.25	1.00	8.00	16.40	8.08	7.20
23.	NRCC-263	Pummelo (*C. grandis*)	840.00	114.40	123.70	6.00	21.00	114.00	9.80	2.00
24.	NRCC-264	Ada jamir (*C. assamensis*)	265.00	83.40	61.60	4.30	9.00	11.00	6.30	6.20
25.	NRCC-265	Jaintia lemon (*C. limon*)	36.00	43.70	40.60	9.00	9.00	18.00	9.00	7.50
26.	NRCC-266	Pummelo (*C. grandis*)	516.00	150.00	105.00	8.90	14.00	102.00	8.20	2.40
27.	NRCC-267	Citron (*C. medica*)	71.33	65.93	47.06	1.60	9.66	17.00	9.20	8.20
28.	NRCC-268	Ada Jamir (*C. assamensis*)	152.00	67.20	63.10	2.35	12.00	41.50	6.10	6.80
29.	NRCC-269	Pummelo (*C. grandis*)	830.00	127.00	135.00	13.00	16.00	106.00	10.00	2.3
30.	NRCC-270	Soh Jhalia (*C. jambhiri*)	90.00	56.00	53.75	2.30	9.50	28.50	6.60	7.68
31.	NRCC-271	Pummelo (*C. grandis*)	716.00	110.70	114.00	9.40	12.00	43.00	9.40	2.60
32.	NRCC-272	Pummelo (*C. grandis*)	420.00	88.00	119.00	8.30	10.00	12.00	8.00	5.5
33.	NRCC-273	Pummelo (*C. grandis*)	890.00	133.00	156.00	24.80	18.00	110.00	9.20	2.90
34.	NRCC-274	Pummelo (*C. grandis*)	418.00	95.60	101.00	10.6	13.00	76.00	8.4	1.50
35.	NRCC-275	Pummelo (*C. grandis*)	638.00	105.70	121.40	18.60	16.00	35.00	8.90	2.27
36.	NRCC-276	Lemon Umroi (*C. limon*)	244.00	125.30	61.60	4.30	9.00	11.00	6.3	6.80
37.	NRCC-277	Rootstock (*C. jambhiri*)	34.00	42.80	38.00	3.20	8.00	1.25	9.25	4.50
38.	NRCC-278	Pummelo (*C. grandis*)	890.00	14.50	145.00	18.40	16.00	72.00	9.20	1.22
39.	NRCC-279	Galgal (*C. peudolimon*)	225.00	94.80	70.40	5.50	11.00	12.50	6.90	7.20
40.	NRCC-280	Assam lemon (*C. limon*)	81.33	77.70	44.93	1.73	10.67	1.00	7.33	7.90
41.	NRCC-281	Pummelo (*C. grandis*)	584.00	111.00	115.00	16.00	14.00	153.00	9.20	2.70
42.	NRCC-282	Assam Lemon (*C. limon*)	92.00	81.76	46.33	1.20	8.33	14.00	6.56	5.25
43.	NRCC-283	Pummelo (*C. grandis*)	822.00	121.30	129.40	11.60	16.00	80.00	9.0	1.4
44.	NRCC-284	Pummelo (*C. grandis*)	1002.00	155.00	128.00	18.20	14.00	28.00	8.40	1.90
45.	NRCC-285	Chambil (*Probable Hybrid*)	81.60	47.92	55.20	3.60	9.60	4.00	8.2	7.04
46.	NRCC-286	Chinara (*C. peudolimon*)	126.67	79.30	57.03	3.30	10.00	3.20	9.90	4.20
47.	NRCC-287	Pummelo (*C. grandis*)	557.58	98.76	120.65	15.2	16.8	56.8	9.50	2.13
48.	NRCC-288	Memon Narang (*C. indica*)	22.00	30.50	40.60	2.05	10.00	10.50	9.98	2.11
49.	NRCC-289	Chinara (*C. peudolimon*)	190.66	84.60	71.03	6.73	11.33	22.20	7.93	3.40
50.	NRCC-290	Memon Narang (*C. indica*)	18.34	23.45	34.24	1.24	10.12	15.46	10.00	2.05
51.	NRCC-291	Pummelo (*C. grandis*)	666.00	101.20	131.00	20.00	18.00	72.00	9.10	1.50
52.	NRCC-292	Khasi Mandarin (*C. reticulata*)	–	–	–	–	–	–	–	–
53.	NRCC-293	Chinara (*C. peudolimon*)	142.00	77.30	61.00	3.167	10.00	3.00	7.6	4.48

Contd.

Table 2. contd.

S. No.	Collector No.	Species/Landraces	Fruit wt (g)	Fruit length (mm)	Fruit breadth (mm)	Peel thickness (mm)	No. of segment	Seeds/ fruit	TSS	Acidity (%)
54.	NRCC-294	Pummelo *(C. grandis)*	588.00	122.00	111.40	10.20	13.00	59.00	9.40	1.22
55.	NRCC-295	Pummelo *(C. grandis)*	682.00	112.20	129.20	14.00	15.00	102.00	8.2	1.50
56.	NRCC-296	Pummelo*(C. grandis)*	790.00	130.00	115.00	9.00	14.00	70.00	9.60	2.30
57.	NRCC-297	Chinara *(C. peudolimon)*	248.00	91.46	72.06	2.43	9.33	31.00	7.40	2.69
58.	NRCC-298	Citron *(C. medica)*	157.33	83.00	60.10	2.80	10.00	55.00	7.60	8.62
59.	NRCC-299	Sharbati lime *(C. limmetta)*	45.50	52.00	39.16	2.125	9.00	23.75	6.65	7.92
60.	NRCC-300	Lemon *(C. limon)*	28.67	42.00	36.03	1.10	8.67	6.00	8.06	8.20
61.	NRCC-301	Citron *(C. medica)*	176.66	98.06	59.36	2.90	10.00	15.00	6.40	8.49
62.	NRCC-302	Pummelo *(C. grandis)*	1204.00	140.00	150.00	11.60	19.00	108.00	9.40	1.50
63.	NRCC-303	Sharbati lime *(C. limmetta)*	32.66	48.45	34.98	0.81	9.83	20.83	6.96	8.45
64.	NRCC-304	Pummelo *(C. grandis)*	114.00	121.80	125.60	14.00	15.00	98.00	9.20	1.80
65.	NRCC-305	Lemon *(C. limon)*	101.50	71.25	50.20	0.925	10.50	32.00	6.85	7.70
66	NRCC-306	Chinara *(C. peudolimon)*	17.70	82.45	71.60	1.75	10.50	27.00	7.10	2.40
67.	NRCC-307	Memon Narang *(C. indica)*	–	–	–	–	–	–	–	–
68.	NRCC-308	Lemon *(C. limon)*	17.60	41.34	29.58	1.36	9.40	15.60	8.48	8.20
69.	NRCC-309	Memon Narang *(C. indica)*	10.50	22.55	29.62	1.60	10.50	9.75	9.40	2.10
70.	NRCC-310	Salonga *(Probable Hybrid)*	563.00	125.00	110.00	4.70	12.00	76.00	6.20	8.94
71.	NRCC-311	Citron *(C. medica)*	64.50	90.50	62.22	4.60	10.50	73.25	6.85	7.50
72.	NRCC-312	Calamondin *(C. madurencis)*	32.32	34.54	39.74	1.56	8.50	6.25	6.50	4.20
73.	NRCC-313	Salonga *(Probable Hybrbid)*	586.00	115.40	105.00	8.70	11.00	10.20	7.0	8.60
74.	NRCC-314	Pummelo *(C. grandis)*	534.00	109.40	117.00	11.20	16.00	13.00	9.2	1.90
75.	NRCC-315	Citron *(C. medica)*	133.33	85.70	53.60	3.63	8.67	23.67	5.27	7.40
76.	NRCC-316	Rough lemon *C. jambhiri*	62.50	51.85	48.90	1.425	9.28	16.25	8.40	5.80
77.	NRCC-317	Pummelo *(C. grandis)*	620.00	112.00	126.00	11.00	15.00	60.00	9.40	2.70
78.	NRCC-318	Karun jamir *(C. aurantium)*	150.24	63.34	68.00	6.25	8.60	6.00	8.00	5.00
79.	NRCC-319	Sweet lime *(C. limettioides)*	98.50	56.20	59.25	3.12	11.00	2.00	6.50	0.34

to deforestation in these area and we observed even farmers cut the trees without any use for fuel etc. It was observed that in Meghalaya most of the citrus species are well maintained in home garden. Therefore, to conserve genetic resources of citrus, emphasis should be given to conserve these species in home garden.

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Isozyme Polymorphism and Genetic Diversity Among *Swertia* Species – Endangered Medicinal Plants of Himalayas

Navin Kumar Verma* and Anil Kumar
Panchwati Greentech Research Society, 11, Rockwood, Darjeeling, West Bengal
** National Bureau of Plant Genetic Resources, Regional Station, Barapani, Shillong-793103, Meghalaya*

Esterase, catalase, peroxidase and malate dehydrogenase banding patterns of five different species of *Swertia* (Family – Gentianaceae) were analyzed in five species *viz.- S. chirata, S. bimaculata, S. cordata, S. peniculata* and *S. petiolata*. All the isozymes tested, showed remarkable variation in electrophoretic banding pattern on PAGE among different species and within species as well. The implications of the findings with reference to genetic diversity and the usefulness of the isozyme technique for germplasm characterization have been discussed.

Key Words: Catalase, Esterase, Malate Dehydrogenase, Peroxidase, *Swertia*

Swertia species (Family – Gentianaceae), included in the endangered medicinal plants list, has being used in the preparation of Ayurvedic medicine since ancient times. Plants are found to grow wild at high altitude (6,000 to 10,000 ft.) in the small pockets of Darjeeling, Kumaoun and Chotanagpur hills of India. Some species of Swertia have also been reported in Nepal, Japan and China. Indian Ayurvedic System of Medicine recommends the use of whole plant in various diseases. Traditionally, *Swertia* species are used as laxative, febrifuge, stomachic, livertonic, anti-malarial, expectorant and bitter digestive tonic. Plant contains various phenyl glycosides namely swertin, swertiomerin, gentiopicroside, amarogentin, ameroswerin, iso-swertianolin, swertianolin and norswertianolin. It also contains oleanolic acid, which has anti-ulcer and analgesic activities and inhibits 5-alpha-reductase which is related to oleopecin. It also has angiotelectase activity (Khetwal and Verma, 1984).

Isozymes are efficient tools for examining genetic variation, because they exhibit Mendelian inheritance, co-dominant expression, complete penetrance and an absence of pleiotropic and epistatic interactions; all of which facilitate genetic analysis (Sanchez *et al.*, 2000). They are used to characterize and identify cultivars, document the parentage of cultivars, or interspecific hybrids and examine the genetic relationship among cultivars in germplasm collections (Bhat and Chandel, 1991; Clement *et al.*, 1997).

Conventionally, different species of *Swertia* are classified on the basis of their morphological features. Existence of various morphological similarities among different species of *Swertia* is a major source of confusion in the collection and classification of genetic variability and maintenance of diverse germplasm collection. Although more than 10 species of *Swertia* are reported, their identity is not well established, which indeed is a pre-requisite for any molecular genetic approach for crop improvement. So far, there is no known report on the biochemical basis of species identification of this plant. This underlines the need to survey variation in the germplasm collections as a function of various isozymes and to depute molecular identification mark, so that the exact nature of species diversification can be demarcated. The present study was undertaken to evaluate the genetic diversity prevalent in five different *Swertia* species and to characterize them using the technique of electrophoresis of isozymes.

Materials and Methods

Five different species of *Swertia*, selected for present studies were – *S. chirata, S. bimaculata, S. cordata, S. peniculata and S. petiolata*. Mature seeds were collected from all the five wild growing *Swertia* species from surrounding areas of Darjeeling near Indo-Nepal border and were multiplied at Panchvati Greentech Research Society (PGRS) Nursery, Darjeeling under uniform environmental conditions. Fresh, soft apical leaves and fibrous roots from one year old plants of each species were collected separately in a closed ice-box. Samples were first powered with the help of pestle and mortar using liquid nitrogen and then homogenized with acetone to remove chlorophyll and other secondary metabolites. The homogenates were filtered through Whatman No.1 filter paper. The residue on the filter paper was homogenized with extraction buffer in a pre-chilled mortar and pestle in a cold room. The sample to buffer ratio was maintained

at 1:3 (w/v) for each sample. The homogenate was then centrifuged at 10,000 rpm for 20 min at 4°C and the clear supernatant was collected. Protein contents of each sample were estimated by Folin – Ciocaltau reagent method (Lowry *et al.*, 1951). The protein contents of each extracts were equalized by the addition of extraction buffer. For each sample 20 µg protein equivalent was loaded on the gel. On completion of electrophoresis, gels were incubated in the respective staining mixtures of different enzymes. After optimum staining, gels were fixed in 7% acetic acid. The relative migration velocity (Rm) was calculated for each band and zymograms were constructed from the Rm values. The process was repeated at least three times to confirm reproducibility of the results.

Results and Discussion

Composition of the extraction buffer is an important consideration to be taken into account for obtaining a better resolution of the enzyme systems. This depends upon various factors like tissues, plant types etc. Each taxon and tissue posses its own set of problems regarding the difficulty of cell breakage and associated problems caused by endogenous tannins, phenols, phenol-oxidases and other cellular constituents. Two more important considerations to be made are adequate cell breakage with minimum time and an optimal tissue to volume ratio. A trial with five different extraction buffers was made for the present study. Buffer containing 0.1M Tris-HCl (pH 7.5), 10% Sucrose, and 0.1% β-merceptoethanol was good for extraction of enzyme from leaf whereas, buffer containing 0.1 M Tris–HCl (pH 7.5), 10% Sucrose, 0.1% β-merceptoethenol and 5% PVP from roots, as these buffers resulted in the maximum number of clear and distinct bands. Zymograms of all the four enzymes drawn to the scale with Rm values are depicted in Fig 1.

Isozyme Polymorphism

Extension studies of polymorphism in isozymes gave reliable estimates of genetic relationship among the garlic genotypes (Bhat and Chandel, 1991). All the four isozymes tested in the present studies showed considerable polymorphism in the number and the pattern of electrophoretic bands produced on the gel.

a) *Esterase (E.C. 3.1.1.2)*

The electrophoretic banding patterns of esterase enzyme were divided into two zones (EstL1 & EstL2) for leaves and three (EstR1, EstR2 & EstR3) for roots. These enzyme zones were governed by two gene loci in leaves and three in roots. EstL1 exhibited dimeric isozymic forms of the enzyme in variable allelomorphic combinations. Lane 1 and 2 species exhibited protein products of gene loci in allelic forms like AA × Aa producing two possible forms of dimeric protein *i.e.* AA (Rm = 0.06) and Aa (Rm = 0.15). Estl1 of lane 3,4 and 5 species exhibited protein product of gene loci in possible two allelic forms in heterozygous conditions *viz.* Aa x Aa, producing 3 possible dimeric forms of proteins *i.e.* AA (Rm = 0.06), Aa (Rm = 0.15) and aa (Rm = 0.2). The banding pattern in EstL2 zone was same as that of EstL1 except for lane 2, clearly indicating that this gene locus also produced dimeric proteins. Lane 2 protein was possibly a product of homozygous gene 'C' as CC (say).

For the enzymes extracted from roots, banding pattern was divided into 3 zones. Zone 1 *i.e.* EstRl of lane 1 and 4 exhibited the same number of bands and banding pattern as that of EstL1 of lane 3, 4 and 5 of leaf samples. Lane 2, 3 and 5 of EstR1 zone exhibited only 2 bands as that of EstL1 of leaves, this was due to two different protein products of alleloform for gene loci 1 *viz.* aa x Aa. EstR2 was completely absent in leaf samples and lane 5 of root samples. This clearly showed an organ specific variation. Lane 1, 2 and 3 of root samples exhibited protein products of allelic combination like Bb × bb, producing dimeric protein products from gene like Bb (Rm = 0.30) and bb (Rm = 0.34). In EstR3 zone, lane 1 and 4 showed 2 bands whereas, lane 2, 3 and 5 showed only 1 band (Fig.1A).

b) *Catalase (E.C. 1.11.1.6)*

Banding patterns obtained from both leaves and the root samples were divided into two zones. All the species showed monomorphic form of proteins in CatL1 zone *i.e.* monomeric protein products of either 'a' or 'A' gene at the same Rm = 0.1. In CatL2 zone, all the species showed dimeric form of protein, but in various combinations of alleles. Bands in lane 1 and 3 of CatL2 zone were the protein products of homozygous gene of different alleles whereas; lane 2, 4 and 5 existed in dimeric protein forms as CC (Rm = 0.35), Cc (Rm = 0.4) and cc (Rm = 0.46).

CatR1 zone of root samples showed a single band in all the species at the same Rm = 0.1 except in lane 5, and exhibited monomorphic form of isozyme. In CatR2 zone, no band was detected in lane 1 whereas,

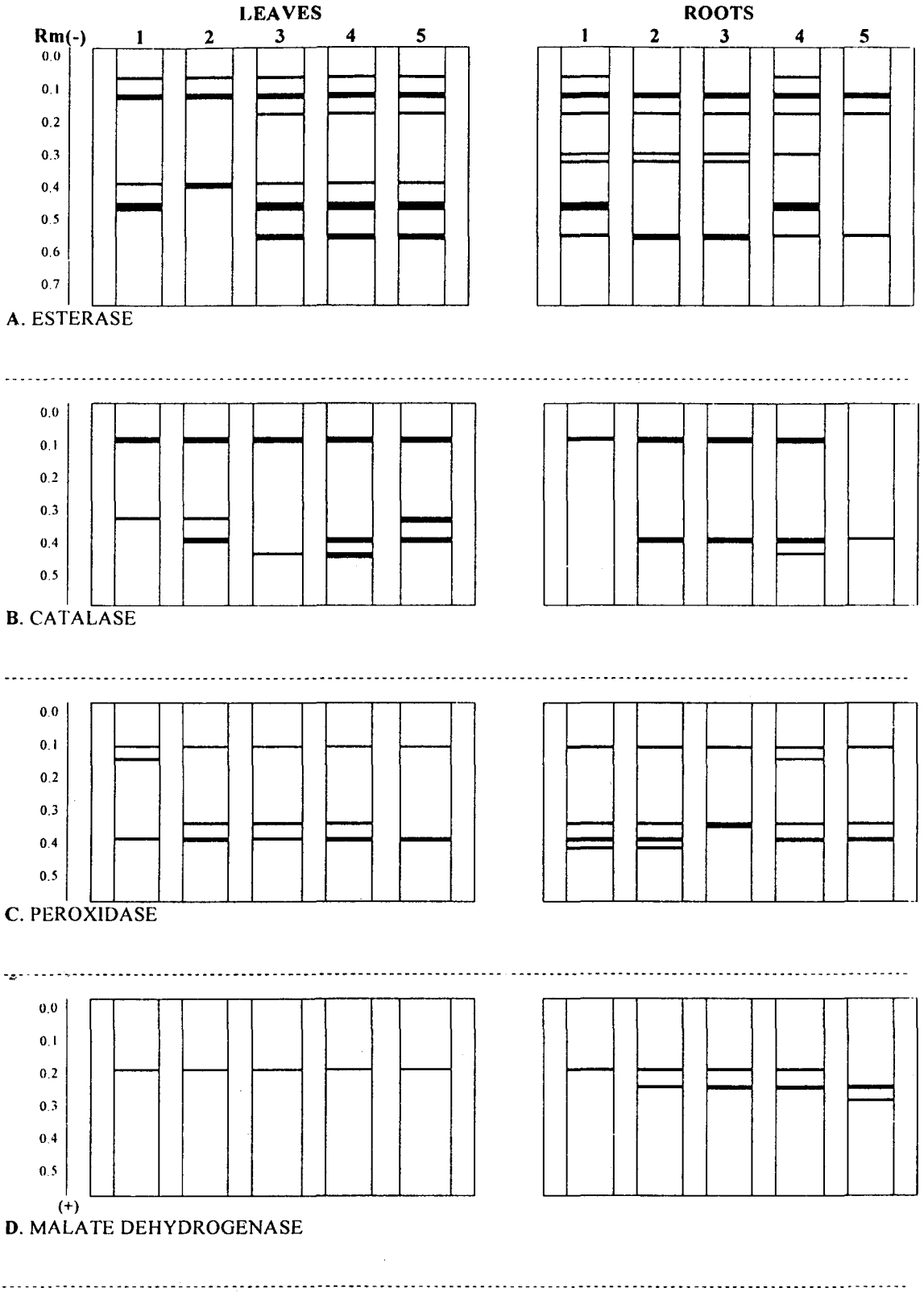

Fig. 1. Isozyme profiles of different Swertia species. (1) S. chitra; (2) S. bimaculata; (3) S. cordata; (4) S. paniculata (5) S. petiolata)

lane 2-5 exhibited dimeric form of proteins; bands of Rm = 0.42 (Cc, say) were present in heterozygous allelic product forms. Apart from this, lane 4 also exhibited protein products of homozygous allelic form at Rm = 0.46 (cc, say) (Fig. 1B).

C. Peroxidase (E.C. 1.11.1.7)

Enzyme from leaf samples of all the species exhibited dimeric nature of proteins in PerL1 zone, which was probably a protein product of homozygous gene AA (say) at Rm = 0.12 whereas, lane 1 had another band at Rm = 0.15 in heterozygous product form of gene Aa (say). In PerL2 zone, all the species showed a common band at Rm = 0.38, a dimeric protein product of heterozygous form of gene Aa (say); while bands at Rm = 0.34 appeared in lane 2, 3 and 4 depicted protein products of homozygous gene AA (say).

Bands appearing in the enzyme of root samples in PerR1 zone of lane 1, 2, 3 and 5 species were similar to that of PerL1 zone of lane 2, 3, 4 and 5 whereas, lane 4 in PerR1 zone was similar to that of lane 1 of PerL1. All the bands in PerR2 zone were similar in banding pattern and Rm values for the species of lane 1, 2 and 5. Protein in PerR2 was dimeric and alleles for a gene 'C' (say) existed in heterozygous from *i.e.* Cc, producing three different combinations to generate three bands. Lane 3 existed in homozygous product form of CC and lane 4 existed in two varied combinations of allele of a gene namely Cc × CC (say), producing two different dimeric proteins (Fig. 1C).

D. Malate Dehydrogenase (E.C. 1.1.1.37)

By comparing leaf and root isozyme banding patterns, we can conclude that all the species exhibiting a single band at Rm = 0.2 were of dimeric protein products of homozygous gene AA (say) in MdhL1 and MdhR1 zone. In MdhR1 zone lane 2-5 showing common banding pattern at Rm = 0.26 existed in hetero-dimeric product of gene form Aa (say); while bands at Rm = 0.2 and at Rm = 0.3 exhibited a homozygous protein product form of gene AA and aa (say) respectively (Fig. 1D).

For unambiguous identification of species, it was essential to use as many isozyme systems as possible for achieving some conclusive results. The data on isozyme systems that were monomorphic in a given set of species was also essential. This study indicated that considerable polymorphism was prevalent for isozymes analyzed. Although, it was not possible to clearly identify any of the species of *Swertia*, based on electrophoretic banding patterns of only one isozyme. However, when complete profile of all the four isozymes from both the root and the leaf were considered, each of the species studied could be distinguished from others.

Further study of the germplasm utilizing some more species with additional isozymes markers and improved separation techniques are necessary to draw a concrete interpretation of genetic variability among different species of *Swertia*.

Acknowledgements

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Incidence of Anthracnose in Indigenous Germplasm of *Dioscorea alata* L.

KI Asha and Maya C Nair
National Bureau of Plant Genetic Resources (NBPGR), Regional Station, Vellanikkara, Thrissur-680654, Kerala

The incidence of anthracnose in indigenous germplasm collection of *Dioscorea alta* L. from different agro-ecological regions of peninsular India were scored on a 0-9 subjective scale under the field epiphytotic condition and percentage infection index was calculated. The study indicated that different morphotypes of *D. alata* varyingly responded to this disease. Analysis of correlation between disease incidence, number of stomata, stomatal size index and average yield/plant showed negative correlation between disease incidence and number of stomata, between number of stomata and stomatal size index and between disease incidence and average yield/plant indicating reduction in yield with the higher incidence of anthracnose. Frequency class distribution of intensity of disease incidence showed a slightly abnormal curve indicating a discontinuous and fragmented genetic variability exisitng in the collection with respect to resistance/susceptibility to anthracnose incidence.

Key Words: Anthracnose, *Dioscorea alata*, Evaluation, Morphotypes

Greater yam or Water yam (*Dioscorea alata* L.) of the family Dioscoreaceae is an important tuber crop under the subsistence farming system in tropics. In India, considerable variability occurs (Muralidharan and Velayudhan, 1985) and both morphological and biometrical techniques have been used by various workers for classification of vast variability (Burkill, 1917). Anthracnose caused by *Colletotrichum gloeosporioides* is a widespread disease in greater yam. This has been reported to have effected yield losses of greater than 80% in cultivars of *D. alata* and found that higher K content and lower content of micro-nutrients are characteristic features of tolerant cultivars (Nwankiti *et al.*, 1987). The intensity of disease incidence varies from place to place and collection to collection in relation to various factors influencing the epidemology of the disease.

To assess the field tolerance of 172 collections belonging to different morphotypes under the field epiphytotic condition, an attempt has been made to observe the intensity of anthracnose incidence for three years in the maintenance plots. The disease incidence has been correlated with average yield/plant and an attempt has also been made to correlate the number of stomata on leaf surface and stomatal size index with the disease incidence of anthracnose in *D. alata* L.

Materials and Methods

Based on the subjective morphotypic classification of *D. alata* L. (Velayudhan *et al.*, 1991), 172 different accessions collected from different agro-ecological regions of Peninsular India were observed in maintenance plots during the years 1994, 1997 and 2000. The experimental plants were raised in augmented design with three controls. Package of practices as recommended by Kerala Agricultural University were followed (Anon. 1996).

During the years 1994 and 1997, the intensity of anthracnose infection was scored on a subjective scale of 0-9 during September-October when the crop attained maximum vegetative growth. During the year 2000, the disease rating was done on the basis of percentage infection of individual accessions using the formula:

$$\% \text{ infection index} = (\text{sum of all disease ratings/leaves}) \times 100/(\text{Total number of ratings/leaves}) \times \text{maximum disease grading}$$

and then converting to 0-9 disease rating scale. Zero indicated complete absence of any symptom of the disease and 9 with an extreme infection of 90-100%. Grades of disease intensity were recorded on the basis of the percentage leaf area covered by the incidence, *viz.* 0% leaf area as 0, 1% as 1, 10% as 3, 25% as 5, 50% as 7 and more than 50% as 9. The observed values were computed and average yield of individual collections were calculated on the basis of mean yield for the three years. Analysis of correlation between disease incidence and yield/plant, between incidence and stomatal size index and between incidence and stomatal number were also studied.

Results and Discussion

The disease incidence scored on subjective 0-9 scale in 172 accessions belonging to 19 different morphotypes of *D. alata* showed the range of incidence from 2-9 with a mean value of 5.47. The yield/plant ranged from

0.58 kg to 4.33 kg with a mean of 2.10 kg. The details of disease incidence, % infection index recorded and average yield of different morphotypes are given in Table 1.

The critical observation on disease incidence in different morphotypes during October, 2000 showed that the maximum susceptibility to the disease was shown by the accessions in M16 (94.24%), M19 (96.22%) and M15 (92.0%). The accessions IC 87387 (13.78%) and IC 266645 (16.22%) in M6 and IC 87372 A (12.28%) in M12 were found comparatively more tolerant to anthracnose. At the same time 100% susceptibility was shown by many accessions in M16, *viz.* IC number 46068, 87330, 87337, 87345 and 87350.

The analysis of correlation between disease incidence and yield/plant showed negative correlation indicating a decrease in yield/plant in relation to increase in disease incidence. The correlation coefficient of -0.217 was significant between disease incidence and yield at 1% level (Table. 2).

Table 1. Anthracnose incidence in *D. alata* morphotypes

Morphotype No.	Morphotype name	No. of accessions	Range	Mean	SD	Mean yield /plant	% infection Index
1.	Chorakkachil	27	3.0-5.67	4.82	0.73	1.98	62.72
2.	Parakkachil	8	3.44-6.0	5.25	0.61	2.35	57.50
3.	Rosakampankachil	5	6.0-6.0	6.00	0	2.20	77.33
4.	Kappakachil	2	5.00	5.00	0	1.83	55.45
5.	Mudiyankachil	1	5.67	5.67	–	1.69	74.22
6.	Thonielayankachil	15	2.67-6.33	4.80	0.98	2.85	52.61
7.	Kuttikkachil	4	3.67 - 5.33	4.75	0.73	2.38	54.95
8.	Muzhayankachil	1	5.67	5.67	0	1.32	67.56
9.	Neelankachil	1	6.0	6.00	0	1.19	68.00
10.	Neendilakachil	4	4.3306.67	5.75	1.55	2.36	66.33
11.	Kaiyyankachil	8	4.0 - 6.67	5.37	0.78	2.22	72.62
12.	Kulambukachil	18	2.67-7.0	4.44	1.31	1.82	51.21
13.	Thalavannankachil	39	3.0-6.67	4.98	0.78	2.21	71.87
14.	Aanikachil	5	2.0-4.67	3.40	1.18	2.24	62.58
15.	Pachamullankachil	3	6.33 - 8.0	7.44	0.96	1.39	92.00
16.	Vattayilakachil	24	4.33 - 9.0	8.25	1.06	1.72	94.24
17.	Chuvannamullankachil	1	4.33	4.33	0	0.89	51.11
18.	Karimpachamullankachil	4	4.67 - 7.67	5.83	1.45	2.34	80.33
19.	Thalikayilakachil	2	7.33-7.67	7.50	0.24	1.98	96.22

Table 2. Analysis of correlation

	Mean yield/plant	No. of Stomata	Stomatal size index	Mean disease incidence
Mean yield/plant	1.000			
No. of stomata	0.332	1.000		
Stomatal size index	0.248	**-0.109**	1.000	
Mean disease incidence	**-0.127**	**-0.219**	0.182	1.000

Fig. 1 depicts the frequency distribution of *D. alata* accessions in 0-9 scale grading and higher frequency was recorded between mean disease incidence of 4 and 6. The incidence of anthracnose in different morphotypes as given in Fig. 2, clearly indicate variations in disease ratings. The same morphotype showed differential responses during the three years and this may be due to the changes in environmental factors that persisted during the cropping seasons over these years. However, morphotypes 15, 16 and 19 had higher incidence in all these years and M6, M8 and M9 had lowest during 1994 and 1997. The presence of the abnormal curve indicates a very complicated nature of the disease incidence. This also shows a high degree of susceptibility of the collections to the disease. It appears that the genetic variability existing in the collections with respect to anthracnose resistance/susceptibility is very discontinuous and fragmented.

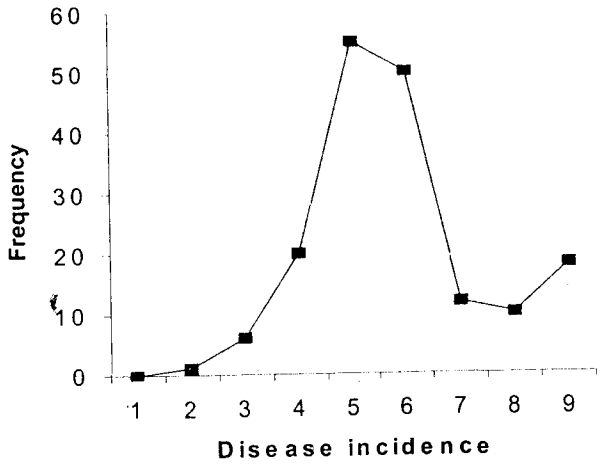

Fig. 1. Incidence of anthracnose in D. alata

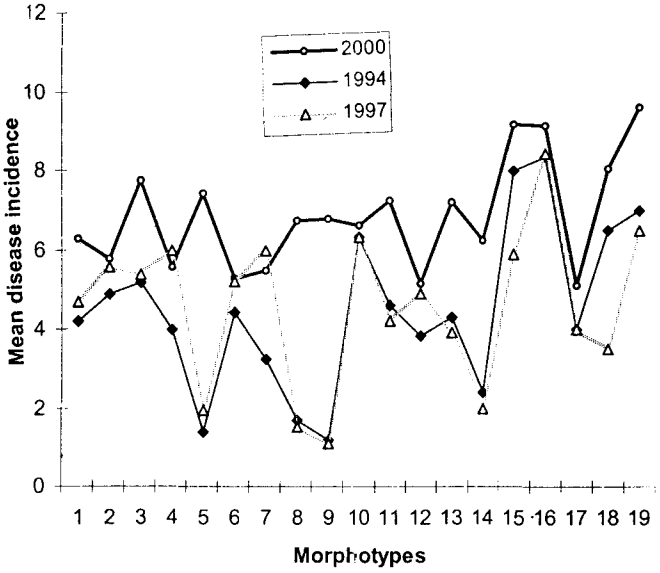

Fig. 2. Differential incidence of anthracnose in morphotypes of D. alata

It has been reported that thicker cuticle, shorter stomata and surrounding cells of stomata with sclerenchyma like cells are characteristic features of resistant lines to Anthracnose blotch disease in *D. alata* (Nwankiti and Okpala, 1984). In the morphotypes of *D. alata* under consideration, leaf epidermal features have earlier been reported (Velayudhan, *et al.*, 1991) and in none of the morphotypes, cell wall thickening or stomata were present on dorsal surface on the leaves. The number of stomata/unit area ranged from 23.70 (M9) to 44.3 (M5). Stomatal size index ranged from 1.42 (M12) to 2.34 (M10). The analysis of correlation between disease incidence, number of stomata and stomatal size index in the morphotypes showed negative correlations between number of stomata and stomatal size index and between number of stomata and mean disease incidence Table 2. This shows that reduction in stomatal size results in increase in the number of stomata/unit area. Even though the number of stomata increases the presence of disease incidence decreases because of the reduction in stomatal size. In conclusion, it was found that there is morphotypic variability in anthracnose incidence and this disease incidence influence the yield significantly.

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Morphological and Fibre Characteristics of *Gossypium arboreum* L. Germplasm Lines Immune to Grey Mildew (*Ramularia areola* ATK.) Disease of Cotton

Punit Mohan, VV Singh and PM Mukewar
Central Institute for Cotton Research, Post Bag No. 2, Shankar Nagar Post Office, Nagpur-440010 Maharashtra

A total number of 1489 *Gossypium arboreum* germplasm lines maintained at the CICR, Nagpur were evaluated for their reaction to grey mildew disease caused by the fungus *Ramularia areola* wherein 7 lines immune (no symptoms) to the disease were identified. These 7 lines namely, Bangladesh (EC 174092) and G-135-49 belonging to race *bengalense* and 30805, 30814, 30826, 30838 and 30856 belonging to race *cernuum* were evaluated for various morphological attributes like leaf, flower and capsule, and the fibre quality parameters *viz.* 2.5% span length, fibre strength, uniformity ratio and fibre fineness for their use in crop improvement programme. The studies revealed wide variability for leaf, flower and boll characters among germplasm lines of race *bengalense* as well as that of race *cernuum*. The genotypes of race *cernuum* indicated high range of variability for economic characters like ginning out-turn (42.15 to 50.75%), boll weight (3.79 to 7.39 g), seed cotton yield (31.71 to 48.32 g/plant) and the fibre properties. The germplasm lines can be used as a good source of resistance for developing resistant cultivars to grey mildew in *arboreum* cotton.

Key Words: *Gossypium arboreum*, Grey Mildew Disease, Race *Bengalense*, Race *Cernuum*

Gossypium arboreum L., commonly known as tree cotton or 'desi' cotton, is a native of India which is cultivated in about 18 % of the total area under cotton, contributes about 10-12 % to the national production. In India, three races of this species *viz. bengalense*, *cernuum* and *indicum* are cultivated.

The grey mildew disease (Dahiya) of cotton caused by the fungus *Ramularia areola* Atk. [*Ramularia gossypii* (Speg.) *Ciferri*] has been reported as a disease of great economic importance in Central India (Sangitrao *et al.*, 1993). Assessment of yield loss has been recorded to the extent of 68 % in intra-*hirsutum* hybrid H4 in disease endemic areas of Vidarbha (Shivankar and Wangikar, 1992). Severe out-breaks of grey mildew have been reported in Central India during the crop seasons of 1958-59 (Gokhale and Moghe, 1965), 1988-89 and 1993-94 (Mukewar *et al.*, 1994). Considering the seriousness of this disease in *G. arboreum* cultivars, a screening programme was undertaken to find out the sources of resistance in the national germplasm collections available at Central Institute for Cotton Research (CICR), Nagpur and as a result seven germplasm lines immune (no symptoms) to the disease were identified (Mukewar *et al.*, 1995).

Present investigation was therefore undertaken to study morphological characteristics and fibre technological properties of the immune lines for improvement of 'desi' cotton.

Materials and Methods

The grey mildew immune lines of *G. arboreum* cotton *viz.* 'Bangladesh' (EC 174902), 'G 135-49', '30805', '30814', '30826', '30838' and '30856' were grown during 1997-98, 1998-99 and 1999-2000 crop seasons at CICR Main Research Farm, Nagpur, and the genotypes were evaluated for morphological characterisation and fibre technological properties. Standard procedures were followed for recording morphological characters and disease reaction. The lint samples of the above designated germplasm lines were analysed at the Central Institute for Research on Cotton Technology (CIRCOT), and for fibre technological properties at the Regional Quality Evaluation Unit, Nagpur.

Results and Discussion

The data presented in Table 1 and Fig. 1 indicate that the germplasm lines Bangladesh (EC 174092), G. 135-49, 30805, 30814, 30826, 30838 and 30856 have a broad variation for leaf, flower and boll characters. Germplasm lines of race *cernuum* had long leaf stalk except accession '30856'. However, genotypes 'Bangladesh' and 'G 135-49' race *Bengalense* had short petiole (Table 1, Fig. 1).

The boll weight is the basic feature of breeding importance in *arboreum* cotton. The race *cernuum* is a low yielder due to less number of bolls/plant. However, high boll weight was recorded in the germplasm

Table 1. Variability in morphological features of germplasm lines of *Gossypium arboreum*

Character	Germplasm Line Identity No.						
	Bangladesh (EC No. 174092)	G-135-49	30805	30814	30826	30838	30856
RACE	*Bengalense*	*Bengalense*	*Cernuum*	*Cernuum*	*Cernuum*	*Cernuum*	*Cernuum*
LEAF							
Leaf shape	5 long narrow lobe, deeply palmate	4-5 small broad lobe, incision; ovate, curvilinear and slightly acute	4-5 long and narrow lobe	5 long and narrow lobe, deeply palmate, rudimentary lobe present	5 elongated lobe, rarely deeply palmate, rudimentary lobe present	5 narrow elongated lobe rarely deeply palmate, rudimentary lobe present	4-5 long narrow lobe, deeply palmate, rudimentary lobe absent
Leaf petiole	Short stalk	Short stalk	Long stalk (upto 8 inches)	Short stalk (upto 3 inches)	Long stalk (upto 8 inches)	Long stalk (upto 8 inches)	Short stalk (upto 3 inches)
Leaf Length (cm)	10.5	7.3	8.5	12.2	Upto 13.0	8.9	8.0
Lobe length (cm)	9.4	4.2	8.4	10.5	12.0	6.2	7.0
Lobe width (cm)	1.8	2.2	1.5	2.3	1.3	2.1	1.5
Lobe sinus (cm)	2.1	2.7	1.6	2.2	1.3	2.1	1.3
Leaf venation	Reticulate and palmate divergent	Reticulate and palmate divergent	Reticulate and palmate divergent	Reticulate and palmate divergent	Reticulate and palmate divergent	Reticulate and palmate divergent	Reticulate and palmate divergent
Leaf surface	Moderately hairy	Moderately hairy	Smooth and glabrous	Smooth	Sparsely hairy	Sparsely hairy	Sparsely hairy
FLOWER							
Pedicel	Long pedicel	Long pedicel	Long pedicel	Short pedicel	Short pedicel	Long pedicel	Short pedicel
Petal colour	Yellow	Yellow-creamy	Yellow	Creamy	Yellow	Yellow	Yellow
Petal claw	Short claw	Short claw	Long claw	Long claw	Short claw	Short claw	Short claw
Petal blotch (length and colour) cm	1.1 (Red)	1.6 (Red)	1.2 (Red)	1.2 (Red)	0.7 (Red)	1.3 (Red)	0.7 (Red)
Petal length (cm)	4.1	3.7	3.5	3.5	3.5	3.9	3.6
Petal width (cm)	2.5	2.7	2.7	3.3	3.5	2.4	3.1
Anther colour and filaments	Yellow and filaments short	Yellow and filaments short	Creamy and filaments short	Creamy and filaments short	Yellow and filaments short	Yellow and filaments long	Yellow and filaments short
Stigma	Short	Short	Short	Short	Short	Short	Short
Bracteole/Bract No.	3, broad	3, small broad	3, small and ovate, acute	3 large and broad	3 large and broad	3 large and broad	3 large and broad
Bract margin and serration	2-4 teeth, margin serrated, apex pointed	2-4 teeth and serrated at anterior part	3-4 teeth and deeply serrated at apex	4-6 teeth, normally serrated at anterior portion	3-4 teeth serrated at apex portion	2-3 teeth normally serrated at apex	5-6 long teeth normally serrated at apex
Bract length (cm)	4.3	2.9	3.7	4.1	5.5	4.4	5.4
Bract width (cm)	3.9	2.5	3.5	3.7	5.0	3.7	4.4
BOLL/CAPSULE							
Boll shape and surface	Conical with tapering end, surface sparsely pitted	Oblong and tapering end, surface densely pitted	Oblong and tapering end, surface densely pitted	Elongated and big, tapering apex, surface sparsely pitted	Elongated and tapering end, surface densely pitted	Big and elongated with tampering end, surface densely	Small conical with pointed appex surface sparsely pitted
Peduncle	Long	Long	Long	Short	Short	Long	Short
Maximum boll length (cm)	4.8	4.4	4.5	5.8	6.2	6.2	5.1
Maximum boll diameter (cm)	3.4	2.5	2.8	3.4	3.6	3.6	2.5
Locule	Trilocular	Trilocular	Trilocular	Trilocular	Trilocular	Trilocular	Trilocular
Locule width (cm)	2.7	2.1	2.4	2.7	2.7	2.7	2.3
Boll volume (cc)	30.2	17.0	15.8	28.0	32.0	38.0	16.0
Boll weight (seed cotton/boll) g	4.10	2.33	3.79	6.11	7.39	7.15	3.98

accessions Bangladesh (4.10 g), 30814 (6.11 g), 30826 (7.39 g) and 30838 (7.15 g) (Table 1).

The capsules/boll are yield contributing components of the cotton plant. Boll volume, boll length and width of septum/locule width showed broad variation between the accessions of race *bengalense* and race *cernuum*.

The seven germplasm lines belonging to *Bengalense* and *Cernuum* races have broad variation in ginning out-turn which ranged between 35.64 to 50.75% (Table 2), however, various researchers in India (Singh and Nandeshwar 1983; Singh and Raut, 1983; Punit Mohan *et al.,* 1992) have reported higher ginning out-turn (GOT) with high seed density/boll and locule in selected genotypes of *cernuum* race.

Improvement in harvest index has played a key role in increasing seed cotton yields in India. Basu and Bhat

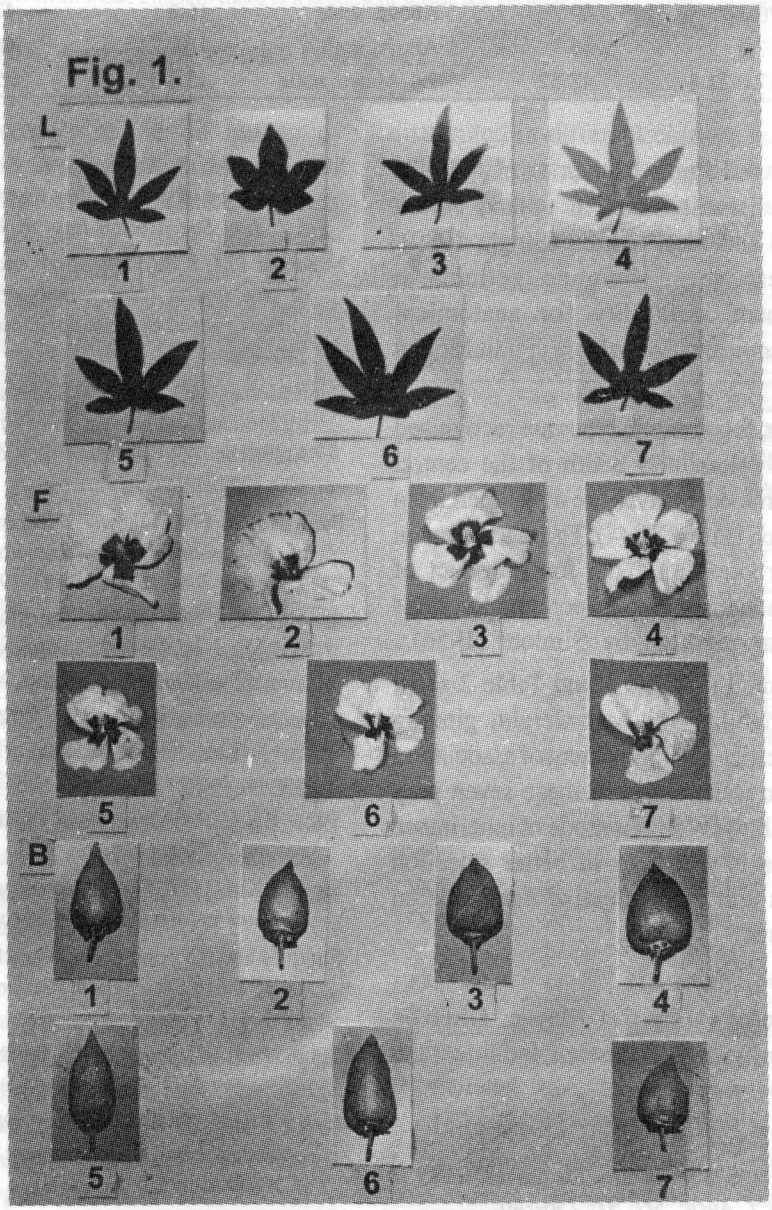

Fig. 1. Variability in morphological features of Gossypium arboreum L. *germplasm lines immune to Grey mildew (*Ramularia areola *Atk.) disease.*

L: Leaf, F: Flower, B: Boll/capsule

1: Bangladesh (EC 174092), 2: G 135-49, 3: 30805, 4: 30814, 5: 30826, 6: 30838, 7: 30856

Table 2. Variability in fibre characteristics and seed yield in germplasm lines *Gossypium arboreum* L.

Germplasm lines	2.5% span length (mm)	Uniformity ratio (%)	Fineness micronaire 10^{-6} g/m	Bundle strength tenacity (g/t 3.2 mm)	Ginning outturn (%)	Seed cotton yield/plant (g)
Bangladesh (EC 174092)	18.4	49	7.4	15.3	44.64	40.73
G 135-49	21.8	50	7.5	19.7	35.64	39.15
30805	20.2	50	7.9	17.9	43.10	44.33
30814	17.5	52	Above scale	16.4	42.75	31.71
30836	18.5	49	Above scale	17.0	50.75	48.32
30838	20.3	50	Above scale	16.6	42.15	44.59
30856	17.0	48	Above scale	16.0	46.10	37.13
Range	17.0-21.8	48-52	7.4-Above scale	15.3-19.7	35.64-50.75	31.71-48.32

(1987), Singh (1988) and Punit Mohan *et al.*, (1992) reported variation in harvest index in upland cotton (*Gossypium hirsutum*) and 'desi' cotton (Singh, 1989). The studies for these seven immune germplasm accessions have shown variability for anatomical structures in race *cernuum* and *bengalense* (Punit Mohan *et al.*, 1997).

In race *bengalense*, four genetic types are recognised on the basis of petal colour and width of the central leaf lobe. However, some derivatives are developed from inter-racial hybridisation (Silow, 1944). The race *cernuum* is really an ecotype representing the final product of localised selection tendencies among perennial cottons in North-East India as reported by Simlote (1956).

The results presented in Table 1 and Table 2 show that a significant variability for boll weight, ginning percentage, fibre properties and their extent of association with several economic traits exists in these seven grey mildew immune lines, however, their mode of inheritance need to be studied as it will help in better selection of parental genotypes and their effective manipulation in breeding programme.

Acknowledgements

The authors are grateful to Dr CD Mayee, Director, CICR, Nagpur, for providing necessary facilities and encouragement.

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Genetic Divergence in Relation to Fatty Acids in Oilseed *Brassica*

P Sinha, SP Singh, Basudeo Singh and ID Pandey
Department of Genetics and Plant Breeding, GB Pant University of Agriculture and Technology, Pantnagar-263145 Uttaranchal

Analysis of genetic diversity using Mahalanobis D^2 analysis was carried out on 50 genotypes of *Brassica* and grouped into 12 clusters. Studies indicated that geographical isolation might not be the only factor causing genetic diversity. Oleic acid and erucic acid were the most potential traits contributing to the total genetic divergence. Clusters III and V were important because they comprised accessions with high and low erucic acid content, respectively and by utilizing accessions from these clusters there is a sufficient scope for varietal improvement through hybridization.

Key Words: *Brassica*, Fatty Acids, Genetic Divergence

To bring genetic improvement in the oil quality of *Brassica,* it becomes imperative to know the fatty acid composition of the available germplasm. There is a great scope to improve oil quality, provided genetically diverse parents are involved in hybridization. In this context, D^2 analysis has been found to be a potent biometrical tool in quantifying the degree of divergence (Rao, 1952) and has successfully been used in many crops (Arunchalam and Bandopadhyay, 1989). The present study was undertaken to estimate the genetic divergence among 50 genotypes of *Brassica* in relation to fatty acid composition.

Material and Methods

Brassica germplasm collection maintained at Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar, were evaluated for fatty acid content. Harvested seeds of 50 genotypes were subjected to fatty acid determination by Gas Liquid Chromatography after converting different fatty acids into their respective esters (Luddy *et al.*, 1968). Genetic divergence was determined using Mahalanobis D^2 statistics (Mahalanobis, 1928, 1949) and the genotypes were grouped into clusters according to Tochar's method (Rao, 1952).

Results and Discussion

The analysis of variance showed highly significant differences between genotypes for each of the seven characters studied. Based on D^2 value the genotypes were grouped into 12 clusters (Table 1). Cluster III has maximum number of twenty-one genotypes followed by cluster I, nine genotypes, cluster V and VI had 4 genotypes each, cluster II and IV had 3 genotypes each and the rest of the clusters had one genotype each. The pattern of clustering demonstrated that the geographical origin of these genotypes is not related to genetic diversity as genotypes of different geographical areas were distributed in different clusters and many genotypes of different geographical origin were grouped into same cluster (Table 1). Similar results have been reported by Alarmelu and Ramanathan (1998) in sesame.

Table 1. Clustering of 50 *Brassica* genotypes

Cluster No.	Number of genotypes	Genotype
I	9	BNLP-3, BNLP-4, BNLP-6, Hyola-401, EC-339014, BNLP-1, EC-339013, BNLP-5, BNLP-10
II	3	BNLP-11, BNLP-12, EC-339017
III	21	Pusa Bahar**, Kranti**, Krishna, Pusa Jaikisan, PRT-1, Kiran**, Vaibhav, RC781, Domo, PT-303, Vardan, YST-151, PYS-841, PYS-842, T-9, Orn. Rai, PYS-843, Pusa Bold, CSR-122 and Zem-2
IV	3	EC-339008, EC-339015, EC-339012
V	4	PRQ-9701, PRQ-9707*, PRQ-9701-2*, PRQ-9705-6*
VI	4	EC-338985, NCN-13, TRL-93, EC-339016
VII	1	EC-339012
VIII	1	PRQ-9705*
IX	1	EC-339001
X	1	EC-339000
XI	1	PRQ-9707-19*
XII	1	Zem-1

* Potential donor for low erucic acid
** Potential donor for high erucic acid

Intra-and inter-cluster distance are presented in Table 2. Intra-cluster values ranged from 0.00 to 19.37. The inter-cluster distance based on mean standard deviation of D^2 value ranged from 20.29 (between VII and IX) to 97.96 (between I and III) and classified into three divergent classes. Highest inter-cluster distance was observed between cluster I and III (97.96) which indicates that these clusters are highly divergent. The next higher

Table 2. Average inter- and intra-cluster in *Brassica* germplasm

Cluster No.	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I	11.005	42.580	97.956	42.740	64.792	68.419	38.406	83.301	55.488	53.186	81.509	74.989
II		17.915	75.415	37.237	39.036	46.332	27.883	50.745	41.482	32.261	68.465	40.233
III			13.062	71.923	79.154	34.927	61.983	68.683	46.713	79.434	87.431	52.820
IV				13.172	66.981	45.756	32.390	70.587	45.704	62.030	87.799	54.208
V					17.778	56.338	48.759	32.333	49.455	27.599	26.725	38.675
VI						19.372	33.418	53.305	23.939	54.004	70.064	35.505
VII							0.000	60.945	20.297	36.650	65.897	46.512
VIII								0.000	56.997	51.638	41.036	23.184
IX									0.000	41.852	61.282	42.935
X										0.000	40.270	48.599
XI											0.000	54.028
XII												0.000

DC1: Highly divergent = >70
DC2: Moderately divergent = 40–70
DC3: Closely related = < 40

distance was observed between cluster IV and XI (87.70). Minimum inter-cluster distance was recorded between cluster VII and IX (20.29) which indicates close relationship between these two clusters. The inter-cluster distance was higher than the intra-cluster distance in all the cases indicating more divergence of genotypes between the clusters. Gondane and Lal (1993) observed similar relationship in okra.

The cluster mean and coefficient of variation also provided an interesting information of the nature of diversity (Table 3). Considerable differences in cluster means occurred for almost all the characters. The coefficient of variation for different characters indicated that major forces of discrimination were erucic acid (93.4%), linolenic acid (76.68%) and ecosinoic acid (71.65%). Maximum cluster mean was observed in cluster number IV for oleic acid content and minimum cluster mean was observed in cluster number IV for linolenic acid. Cluster mean for erucic acid content ranged from 1.71 (cluster V) to 44.45 (cluster III). In order to enhance the usefulness of mustard oil as a food crop, a further increase in level of oleic acid along with decreases in erucic and linolenic acid is desirable. Reduction of linolenic acid is necessary to improve the shelf life of the mustard oil while reduction in erucic acid decreases blood cholesterol. (Renard and Mc Gregor, 1976). Oleic acid is the common precursor of erucic acid as well as linolenic acid. Increased oleic acid concentration and its further desaturation gives rise to higher concentration of linoleic linolenic acid. On other hand erucic acid accessions

Table 3. Mean cluster values for different traits in *Brassica*

Cluster	Palmitic acid 16:1	Stearic acid 18:0	Oleic acid 18:1	Linoleic acid 18:2	Linolenic acid 18:3	Eicosenoic acid 20:1	Erucic acid 22:1
I	4.942	1.018	78.194	10.389	0.422	0.443	4.652
II	4.965	0.423	43.917	23.917	7.017	13.220	6.162
III	3.688	0.796	13.101	17.546	13.264	7.718	44.446
IV	4.318	0.660	61.243	5.110	2.893	12.097	14.182
V	6.117	0.606	27.726	34.552	21.950	7.586	1.714
VI	5.207	0.694	30.710	18.400	10.194	7.914	30.011
VII	3.615	0.550	50.165	18.010	4.000	4.600	20.885
VIII	7.945	0.965	13.550	31.100	29.440	15.390	2.125
IX	2.920	0.625	40.080	18.990	9.500	1.005	28.510
X	4.345	0.755	33.770	35.980	8.525	6.005	83.335
XI	6.410	0.230	19.335	41.895	30.240	0.735	2.030
XII	4.460	0.550	18.160	28.895	20.850	16.685	12.520
CD	1.382	0.217	20.245	10.745	10.115	53.508	13.660
CV%	37.059	33.020	56.370	45.900	76.680	71.550	93.420

are important for industrial purpose. Cluster III, V, VIII and XI could be regarded as useful sources of genes for different traits and some of the promising germplasm accessions for desired traits are mentioned in Table 1.

Contribution of individual character towards divergence (Table 4) revealed that maximum contribution to total divergence is recorded by oleic acid (45.39%) and erucic acid (33.47) followed by linolenic, ecosinoic, palmitic and stearic acid in decreasing order. In view of considerable genetic diversity in *Brassica* germplasm there is ample scope for varietal improvement through hybridization.

Acknowledgement

The authors are grateful to the CSIR for providing Senior Research Fellowship to Mr ID Pandey.

Table 4. Contribution of each character to genetic divergence

Cluster	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid
No. of time appearing first in ranking	6	5	556	75	87	86	410
% Contribution	0.49	0.41	45.39	6.12	7,10	7.01	33.47

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Investigation of Costus Root Oil Composition

CS Raghav, Poonam Suneja, Jitendra Mohan, VK Srivastava, Mahendra Singh and SN Naik*
National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012
** Indian Institute of Technology, New Delhi*

A study was undertaken to collect plants occurring wild in the forest for their chemical examination so that possibilities of their domestication and utilisation as a quality product in the organised sector could be visualised. In this context, the roots of *Saussurea lappa* collected from Chamoli district of state of Uttaranchal, India were subjected to extraction for their essential oil and its composition. It was observed the cold percolation with diethyl ether optimal quantity of oil. The analysis of oil obtained by cold percolation revealed that there was one chemical component, dehydrocostus lactone, which is thermolabile and decomposes fast during hydro-distillation. This particular component was isolated and its structure was determined with IR Spectroscopy and NMR. Essential oil obtained from costus root is not only important in medicine, but it has potential to the utilised in perfumery industry.

Key Words: Costus, Dehydrocostus lactone, Essential oil, *Saussurea lappa*

Costus (*Saussurea lappa*) plant is a tall, sturdy, herbaceous, perennial up to 2 metre high. It is found in North Western Himalayas. Since ancient times, the oil of costus root has been used against several kinds of diseases. In action, it is anodyne, antiarthritic, antiseptic, aphrodisiac, astringent, carminative, diaphoretic, deodorant, disinfectant, diuretic, emmenagogue, expectorant, febrifuge, spasmodic, stimulant, tonic etc. (Chauhan, 1999). Dehydrocostus lactone, a sesqui-terpene, is an important compound found in the roots of this plant. This inhibits the expression of inducible nitric oxide synthase and TNF-alpha in LPS- activated macrophages. Thus dehydrocostus lactone may be a petential compound for the development of new drugs to treat endotoxemial accompanied by the over production of NO and TNF-alpha (Lee *et al.*, 1999). Large quantities of costus root are exported from Kashmir to China and Japan for use in temples and to Europe for oil distillation purpose (Gulati, 1982). Costus resinoid essential oil is in great demand as a fixative in perfumery industry.

Materials and Methods

The roots of costus (*Saussurea lappa*) were collected from Chamoli district of Uttar Pradesh in North India. The powdered roots (25 g) mixed with 10gm silica gel (to increase surface area for better extraction) were extracted (cold percolation) with diethyl either, petroleum ether, benzene and methanol (350 ml each) consecutively (the previous solvent was completely removed before using next solvent) in a glass percolator (ID 3 cm) with a flow rate of 0.6 ml per minute. The extracts were cool concentrated and yield of oleoresins were recorded. Diethyl ether extract oleoresin was subjected to Thin Layer Chromatography (TLC). The solvent for TLC chamber was used as mixture of toluene and ethyl acetate in 93:7 proportion for detecting the number of compounds. The oil was separated from this oleoresin by chilling method and hydro distillation separately, which was subjected to GLC (Model-Perkin Elmer Auto System equipped with capillary column) for comparative composition. A third method using Super Critical Fluid Extraction (SCFE) technique was also used for the oil extraction (Gas CO_2, pressure 70 bar, T10°C). A single compound was also isolated through preparative TLC technique from the ether extracted oil, which was further analysed on FTIR and NMR (FTIR- model Nicolet Impact-400, No. of scan-32, Resolution-4, Apodization-happ-genzel, detector-DTGS polyethylen, beam splitter-KBr). NMR (model-Em 360L, Frequency-60 MHz, magnetic field strength 1.4 tesla, sample revolution-42 rps) for its identification.

Results and Discussion

The yield of oleoresin obtained with cold percolation with diethylether was recorded as 6.68%. The yield obtained by subsequent extraction of the sample with petroleum ether, benzene and methanol (Table 1) indicates that a total of 22.87% oleoresin was obtained from the sample, highest being with the methanol extract (15.95%).

The oil obtained from cold diethylether percolation was brownish yellow with a peculiar iong lasting odour. The TLC of the oil resolved various compounds. The oils obtained from cold percolation and hydro-distillation were tested on GLC separately for

Table 1. Fraction (cold percolation) yield of oleoresin and oil with various solvents

Fraction	Solvent	Yield of oleoresin		Oil yield (%)
		(g)	(%)	
Ist fraction (350 ml)	Diethyl ether	1.6716	6.68	1.16
II fraction (350 ml)	Petroleum ether	0.0133	0.05	0.04
III fraction (350 ml)	Benzene	0.0475	0.19	0.10
IV fraction (350 ml)	Methanol	309871	15.95	0.25
SCFE) (diethyl ether fraction	–	–	–	4.23

comparison. It was found that some peaks were missing in the oil obtained from hydro-distillation (Figs. 1 and 2), which indicated the presence of some thermolabile compound in the oil.

The essential oil was put on two different TLC plates, one preparative and the other analytical (solvent toluene: ethyl acetate, 93:7) and major spots were observed in the UV light. One plate was sprayed with dragan droff

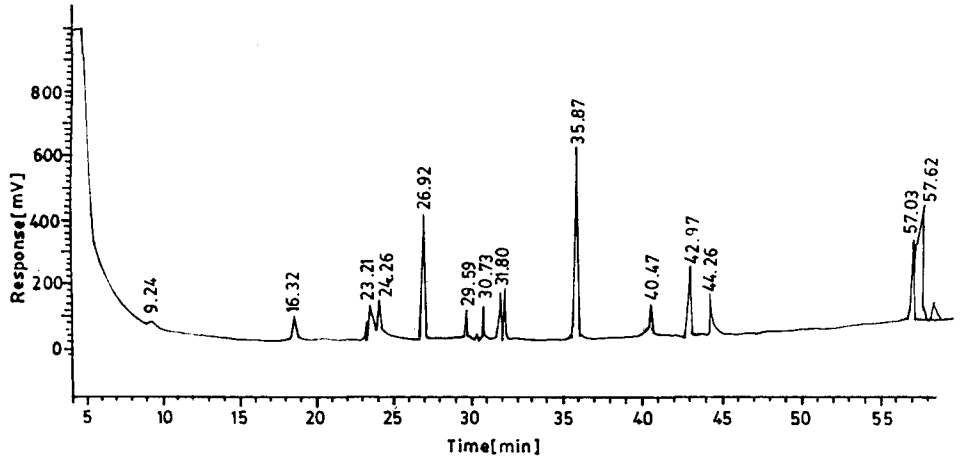

Fig. 1 Gc Graph of cold percolated oil

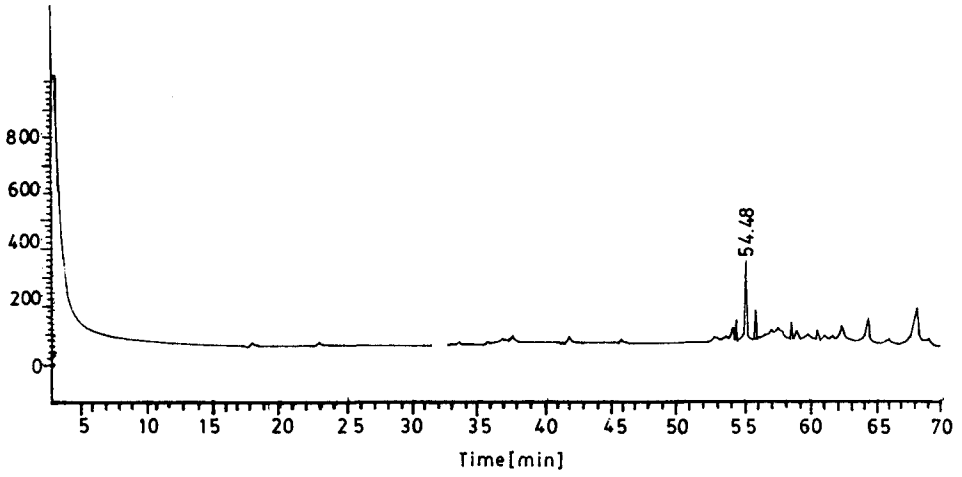

Fig. 2 Gc Graph of cold percolated oil (after heating)

reagent. On analytical plate the lower most portion (Rf value 0.03) gave a violet colour indicating the presence of an alkaloid. Subsequently H_2SO_4 was sprayed on the plate. Charring at some places indicated the presence of other compounds. The second preparative plate was also examined in UV light. A cluster of few spots was observed between Rf 0.3 to 0.5. Above this cluster a single spot was seen at Rf 0.81. The spot material on plate was scraped separately. On physical examination it was found the spot at Rf 0.81 has a pleasant smell resembling to the natural smell of its parent root material. This was filtered and purified and subjected to IR and NMR, which confirmed the compound as dehydrocostus lactone, which was first time isolated by Ukita (1939). Structure interpreted from FTIR (Fig. 3) and NMR (Fig. 4) is given in Fig. 5 as also reported by Mathur *et al.*, (1965). The characteristic peculiar smell of the oil was mainly due to this compound as the remaining part of the preparative TLC was also isolated, which had no smell.

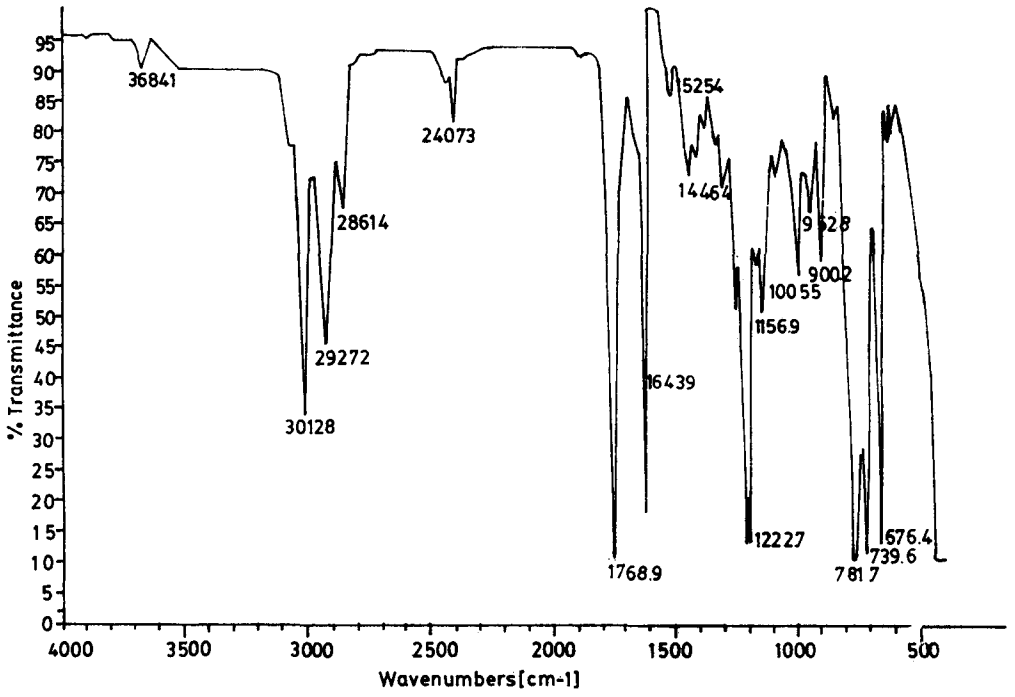

Fig. 3 FTIR spectra of dehydrocostus lactone

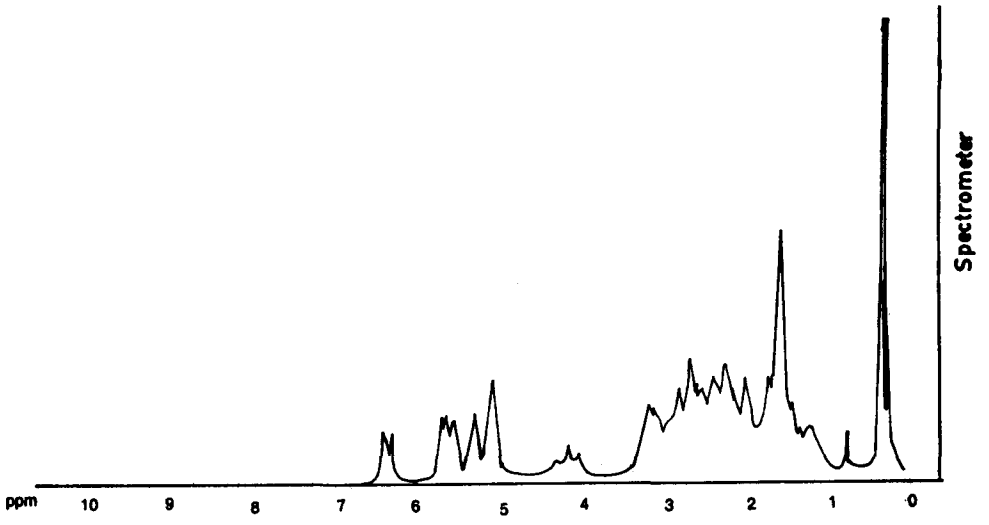

Fig. 4 NMR spectra of dehydrocostus lactone

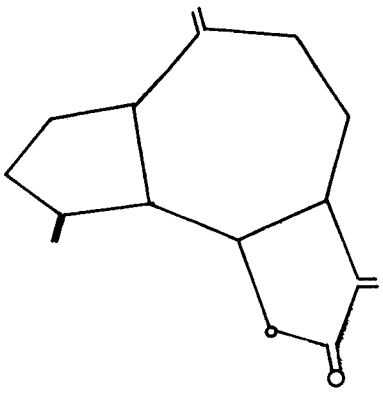

Fig. 5 Spectra of dehydrocostus lactone

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Horticultural Biodiversity in Santhal Parganas

Vishal Nath, Baleshwar Singh* and Mathura Rai
Central Horticultural Experiment Station, Indian Institute of Horticulture Research, Ranchi-834010, Jharkhand
** National Bureau of Plant Genetic Resources (NBPGR), New Delhi-110012*

Santhal Pargana region in Bihar is characterized by tribal community and natural forests. The area receives medium-high rainfall (1200-1600) mm/annum) distributed between the month of June of September with mild temperature (30°-36°C) which favours growth of a number of horticultural crops of major and minor importance. Most of these crops are cross pollinated and show natural regeneration, leading to considerable variability accumulation in many fruits. The identification and collection of economically important genotypes of jackfruit, custard apple, jamun, tamarind, bael in this season was carried out. Biodiversity areas and collection routes are enumerated. A number of promising collections for various traits in different fruit crops are described.

Key Words: Biodiversity, Fruit crops, Santhal Pargana

The plateau region in Bihar is spread over a large area covering the southern and south-eastern part of the state. The region is dominated by tribal communities like Santhals, Mundas, etc. and has been named on the basis of their predominance. Santhal Pargana consists of six tribal districts *viz.* Dumka, Godda, Pakud, Sahebganj, Deoghar and Giridih. The region is situated between 23°50' to 25°15' N latitude and 25°53' to 87°51' E longitude with elevation ranging between 450-1000 m above mean sea level. It is endowed with mild, hot and humid climatic conditions which favours a large number of indigenous and few exotic naturalised plant species to grow. This constitutes the wide genetic base of many important horticultural species. The prominent fruit species exhibiting rich genetic diversity in the region are *Artocarpus, Mangifera, Buchanania, Syzygium, Aegle, Emblica* (indigenous) and *Annona, Tamarindus, Carica, Phoenix* (exotic, naturalised). Climatically, the region has been classified as semi-humid sub-tropical which receives rains throughout the year with maximum concentration during June to September. The average annual rainfall of the region varies between 1200-1600 mm. The region has red-yellow and light gray category of soils which are acidic in nature and contains low organic matter with poor water holding capacity. Undulating topography and slopes invariable coupled with high rainfall aggravates the problem of soil erosion in the region. High rainfall and warm climate has built up a thick forest cover on about 30% land area in the region. These forests have high degree of genetic variability in fruits, vegetables and medicinal plants which require due attention for their conservation. As a predominately tribal area, the main occupation of people in Santhal Pargana is agriculture. However, they are also utilising the natural horticultural species for their livelihood and thus, promoting their *in situ* conservation. Considering the immense potential of various horticultural species, a mission mode project has been launched to collect and conserve the horticultural biodiversity of the region under the National Agricultural Technology Project.

Materials and Methods

A joint exploration was undertaken in collaboration with Central Horticultural Experiment Station (IIHR), Ranchi and National Bureau of Plant Genetic Resources, New Delhi during second fortnight of May, 2000. A total of 9 districts *viz.* Deoghar, Banka, Dumka, Pakud, Shebganj, Bhagalpur, Godda, Dhanbad, Bokaro and Hazaribagh (Santhal Parganas) and adjoining areas were surveyed for various fruit species. Genetic variability of five fruit species (*Artocarpus, Syzygium, Aegle, Tamarindus* and *Annona*) were critically studied and promising genotypes identified for various horticultural traits. High, medium and low degree of diversity in these species were categorised depending upon the population of species at particular diversity site. Individual plants were selected on the basis of useful horticultural traits in each species. Detailed observations were recorded in jack fruit, which is one of the most important multipurpose plant of the region. Quantitative observations on average number of fruits, fruit length, fruit circumference and fruit weight in each genotype were recorded. The qualitative observations on plant growth habit, foliage characters and appearance of the fruits were also recorded. Information pertaining to ethno-botanical knowledge, their utilization pattern, Indigenous Technical Knowledge (ITK), pattern of cultivation and conservation, were documented.

Results and Discussion

The Santhal Pargana region has rich genetic stock of jack fruit, custard apple, tamarind, bael, jamun and wild dates. Rai and Gupta (1996) have also mentioned the natural biodiversity of these species in plateau region of Bihar. During the exploration, high degree of diversity in various horticultural crops have been observed at different sites. Extent of diversity in some important crops have been presented in Table 1. Jack fruit has low to high degree of diversity at all the sites in the region. Custard apple is concentrated mainly in Dumka, Pakur and adjoining areas of Sahebganj and Godda. *Jamun, bael,* tamarind and *chiroji* have sporadic and localised distribution in the region (Table 3). Variability pattern of the germplasm and its various uses by the local community are described below:

Table 1. Diversity distribution of fruit crops in Santhal Parganas

Name of village	Block/District	Available fruit plant				
		Jackfruit	Bael	Tamarind	Custard Apple	Jamun
Jogia	Mohanpur, Deoghar	++	+	+	-	-
Narayanpur	Jaipur, Banka	++	-	-	+	-
Naya Lohmadwa	Sariya Hat, Dumka	+++	+	+	-	+
Nawa Deeh	Sariya Hat, Dumka	++	+	-	+	++
Nani Hat	Jama, Dumka	++	-	-	+	+
Baraplasi	Jama, Dumka	+++	+	+	+	-
Kukurtava	Kukka, Dumka	+++	+	++	++	+
Negbeel	Dumka, Dumka	++	++	-	+	++
Jhurkund	Kumka	++	-	++	++	+
Kathikund	Dumka	++	-	+	++	-
Gopi Kandar	Dumka	+	-	+	+	++
Durgapur	Di,la	++	++	++	++	+
Amrapada	Pakud	++	-	++	+++	++
Fathepur	Pakud	+	-	+	++	++
Salpatra	Pakud	+	-	+	+++	+
Paderkola	Pakud	++	-	+	++	+
Surajpada	Littipad, Pakud	++	+	++	+++	+
Hiranpur	Littipad, Pakud	+++	-	-	++	-
Dharmapur	Littipad, Pakud	+++	++	++	+	++
Barhet	Littpad, Pakud	++	-	-	++	++
Mirzapur	Sahebganj	+	++	+	-	++
Mirzachawki	Bhagalpur	+	++	-	-	-
Pathargama	Godda	-	-	++	-	+
Bhatdeeha	Godda	++	-	+	-	+
Marcee Hospital	Pauraiyahat, Godda	+++	-	-	-	-
Kudwa	Dumka	++	-	-	+	-
Gujisemal	Dumka	++	-	++	++	-
Pattabadi	Dumka	++	+	-	++	++
Kushpahadi	Dumka	+++	++	++	++	++
Barmasia	Shikaripada, Dumka	+++	++	+	+++	+
Shikaripada	Dumka	++	-	+++	++	++
Nishchintpur	Dumka, Dumka	++	+	-	++	-
Mashalia	Mashalia, Dumka	++	-	+	+	+
Shikarpur	Dumka	-	-	+	+++	-
Heraljori	Dumka	++	-	+	++	-
Tehsuriya	Kundhat, Dumka	+	+	++	-	++
Sudarpur	Nala, Dumka	+	+	-	-	-
Guhiajori	Nala, Dumka	+	-	-	-	-
Novadeeh	Jamtara, Dumka	+++	-	-	++	+
Ratanpur	Govindpur, Dhanbad	++	+	-	-	-
Balideeh	Bokaro	+	-	-	-	-
Ramgarh	Hazaribagh	+	-	+	-	+

+++ =High variability; ++ =Moderate variability; + = Low variability; - = Variability not observed

Biodiversity of Jack Fruit

Jack fruit is the most common and wide spread species in Santhal Pargana region. Plant population per unit area of the species is inversely proportional to the overall development of the particular site. The sites with well developed agriculture have low plant population as in case of Gopi Kandar (Dumka), Salpatra (Pakud), Mirzapur (Sahebganj), Mirzachawki (Bhagalpur) and Balideeh (Bokaro). In contrast, area having mixed cropping pattern, and closer to the consumption/disposal centers (towns), the plant population of jack fruit is very high. For example, Naya Lohmadwa which is located about 20 km from Deoghar has high concentration of jack fruit. This area has on an average 10-15 jack fruit plants per hectare particularly on the bunds of fields. Usually mixed cropping with maize, turmeric, pigeon pea and some upland paddy is commonly practiced. During the exploration it was observed that the barren and uncared lands also have low population of jack fruit trees *e.g.* Tehsuriya and Sundarpur (Dumka) and Ramgarh (Hazaribagh).

The jack fruit is a cross pollinated species and predominantly propagated by seed, which has resulted in high range of genetic diversity. Melanha (1998) mentioned that jack fruit is an ancient fruit of India which grows throughout the tropics on variety of soils. Rana (1993) mentioned rich diversity of jack fruit in India. Arora (1998) also reported diversity of *Artocarpus heterophyllus* in Western Ghats and central-east tribal region of the coutry. On the basis of maturity period, the jack fruit genotypes have been locally classified as *Jethua* types (matures early) and *Sawania* type (late maturing) apart from very late type called *Bhadonha.* Considering the use options, the jack fruit genotypes have been grouped as table type (ripe fruits) and vegetable and pickling type (raw). Diversity was observed with respect to tree characters, fruiting behaviour, fruit characters and yield performance among the genotypes. Upright to spreading trees with dense to sparse foliage have been observed in the region (Table 2 and 2a). The fruit length has been recorded between 20.8 to 80.0 cm, circumference from 19.5 to 78.5 cm and fruit weight from 3.5 to 4.50 kg among the identified genotypes. Characters like fruit colour, shape, uniformity and pattern of tubercles varies among genotypes. Yield/tree is a factor of fruit number and weight of fruits on a particular tree which also varies among the genotypes.

Biodiversity of Custard Apple

Natural population of custard apple is available thought the Santhal Pargana. However the high concentration has been observed at Shikaripada, Shikarpur and Durgapur in Dumka district and Amrapada, Salpatra, Surajpada in Pakud district. In general, the plants are more prevalent in undisturbed forests and groves. Population density of custard apple varies from site to site in explored areas. In forest area of Shikaripada as many a 70-80 natural plants per hectare of custard apple were seen. The plant develops multistem which flowers during April-May. Ripening of fruit takes place during September-October. Some early types ripens even during mid of August. Rai and Gupta (1996) mentioned diversity distribution of *Annona* in eastern peninsular region of country. Arora (1985) and Pareek and Sharma (1993) mentioned variability of custard apple in India. Pareek and Vishal Nath (1996) enumerated that although custard apple was introduced to India but it has developed considerable variability in warmer and humid part of the country.

Biodiversity of Bael

Santhal Pargana region has sporadic distribution of bael (*Aegle marmelos* Corr.). *Bael* is an indigenous fruit having medicinal importance and its rich distribution has been recorded in warmer parts of the country particularly in Vindhayan hills (Rai *et al.,* 1991; Pareek *et al.,* 1998). In the present exploration, scattered population of bael was noticed in all the tribal districts of the region. Among the available diversity, plants have shown variability with respect to tree height, foliage pattern and fruit characteristics. A total of 7 genotypes have been collected from different exploration sites (Table 2). Rai *et al.,* (1991) and Rai and Gupta (1996) reported high degree of phenotypic variability in wild *bael* available in north-central and eastern parts of the country.

Biodiversity of Jamun

Jamun (*Syzygium cuminii*) is a native fruit of India (Sham Singh *et al.,* 1967), possesses high medicinal value and grown all over the country (Rai and Gupta, 1996). However, Indo-Gangetic plain of north and eastern India has maximum diversity (Irulappam and Anbu, 1994). Santhal Pargana region which is very close to gangetic plain of Bihar also has considerable plant population and rich diversity in tree and fruit morphological characteristics in jamun. During the present survey, 3 genotypes having larger fruit, sweet pulp and more edible

Table 2. Tree characteristics and fruit appearance of jackfruit selections from Santhal Parganas

Accession (collectors no.)	Plant growth habit	Canopy characters	Foliage density	Fruit colour	Fruit shape	Unifo -rmity	Distribution of tubercles
VN/BS-J0	Upright	Less spreading	Sparse	Yg	Eo	U	Ts
VN/BS-J1	Vigorous	Highly spreading	Sparse	Yg	R	U	Ts
VN/BS-J2	Vigorous	Highly spreading	Dense	G	R	U	Td
VN/BS-J3	Vigorous	Highly spreading	Dense	Yg	O	U	Ts
VN/BS-J4	Less vigorous	Less spreading	Sparse	G	O	U	Ts
VN/BS-J5	Vigorous	Highly branched	Dense	Yg	E	U	Td
VN/BS-J6	Vigorous	Branched	Dense	G	O	U	Td
VN/BS-J7	Vigorous	Branched	Mid-dense	G	L	U	Td
VN/BS-J8	Less vigorous	Med. Spreading	Mid-dense	G	L	U	Td
VN/BS-J9	Less vigorous	Less branched	Sparse	G	Ml	U	Ts
VN/BS-J10	Less vigorous	Branched	Dense	G	L	U	Ts
VN/BS-J11	Upright	Less spreading	Dense	G	L	U	Ts
VN/BS-J12	Vigorous	Spreading	Dense	Yg	L	U	Ts
VN/BS-J13	Vigorous	Spreading	Dense	Yg	L	U	Ts
VN/BS-J14	Less vigorous	Med. Spreading	Mid-dense	Yg	R	U	Ts
VN/BS-J15	Vigorous	Highly spreading	Dense	Yg	O	U	Ts
VN/BS-J16	Vigorous	Highly spreading	Dense	Yg	O	U	Ts
VN/BS-J17	Less vigorous	Spreading	Dense	Yg	O	U	Ts
VN/BS-J18	Vigorous	Less branched	Sparse	Yg	R	U	Ts
VN/BS-J19	Vigorous	Spreading	Dense	G	L	U	Td
VN/BS-J20	Upright	Med. Spreading	Dense	G	L	U	Td
VN/BS-J21	Vigorous	Spreading	Mid-dense	Yg	R	U	Ts
VN/BS-J22	Vigorous	Less spreading	Dense	G	L	U	Ts
VN/BS-J23	Vigorous	Less spreading	Dense	G	L	U	Td
VN/BS-J24	Less vigorous	Less spreading	Mid-dense	Yg	R	U	Ts
VN/BS-J25	Vigorous	Highly spreading	Mid-dense	Yg	R	U	Ts
VN/BS-J26	Med. vigorous	Med. Spreading	Dense	Yg	MI	U	Ts
VN/BS-J27	Med. vigorous	Med. Spreading	Dense	Yg	L	U	Ts
VN/BS-J28	Less vigorous	Med. spreading	Sparse	Yg	L	U	Ts
VN/BS-J29	Vigorous	Spreading	Mid-dense	Yg	R	U	Ts
VN/BS-J30	Vigorous	Spreading	Dense	G	R	U	Ts
VN/BS-J31	Less vigorous	Less spreading	Sparse	Yg	O	U	Ts
VN/BS-J32	Tall	Less spreading	Sparse	Yg	O	U	Ts
VN/BS-J33	Tall	Less spreading	Dense	G	O	B	Td
VN/BS-J34	Vigorous	Highly spreading	Dense	Yg	L	U	Ts
VN/BS-J35	Vigorous	Highly spreading	Dense	Yg	O	U	Ts
VN/BS-J36	Med. vigorous	Less spreading	Mid-dense	Yg	O	U	Ts
VN/BS-J37	Med. vigorous	Spreading	Dense	yg	O	U	Ts
VN/BS-J38	Less vigorous	Less spreading	Dense	Yg	O	U	Ts
VN/BS-J39		Spreading	Sparse	Yg	Eo	U	Ts

Fruit Colour : Yg-Yellowish green, G-Greenish
Fruit Shape : Eo-Elongated oblong, R-Roundish, O-Oblong, E-Elongated, L-Long, MI-Medium long
Uniformity : U-Uniform, B-Brown
Distribution of tubercles : Ts-Tubercles spreading, Td-Tubercles dense
* Bunching type; *** Precocious and prolific bearer; ^ High bearing, early type fruiting in bunch; [B] Late maturing type

portion have been identified. In general, the region does not have systematic plantation of jamun but its plants are quite visible at community lands, road sides and in house yards.

Biodiversity of Tamarind

Tamarind (*Tamarindus indica* L.) is a hardy fruit tree which flourishes well under extremes of odd conditions like degraded, eroded, gravely land with uneven rainfall. Although it is a naturalised crop in India but wide variability has been reported in different parts of the country (Pareek and Vishal Nath, 1996). In the surveyed area sporadic population of tamarind have been observed. Old seedling plantations along the road side are quite common. Being cross pollinated (Thimmaraju *et al.*,

Table 2(a). Fruit characters of jack fruit genotuypes from Santhal Parganas

Genotype Collector (nos.)	Average fruit number	Fruit length(cm)	Fruit circumference (cm)	Average fruit weight (kg)
VN/BS-J1	23.00	47.50	55.00	30.67
VN/BS-J2	125.00	40.00	65.70	35.00
VN/BS-J3	113.00	32.50	48.00	13.50
VN/BS-J4	48.00	45.00	53.50	32.50
VN/BS-J5	48.00	38.50	54.00	16.50
VN/BS-J6	84.00	43.00	57.30	36.00
VN/BS-J7	83.33	45.43	52.40	27.50
VN/BS-J8	62.67	50.50	50.70	30.00
VN/BS-J9	73.67	48.00	43.60	15.50
VN/BS-J10	44.67	40.50	38.00	20.00
VN/BS-J11	53.67	45.50	38.50	30.50
VN/BS-J12	22.33	40.50	30.00	20.50
VN/BS-J13	125.00	52.30	57.80	32.80
VN/BS-J14	73.67	60.00	59.50	35.00
VN/BS-J15	17.33	37.20	38.00	27.30
VN/BS-J16	158.33	60.80	59.70	28.00
VN/BS-J17	43.67	61.20	58.00	32.00
VN/BS-J18	113.67	30.50	28.70	15.00
VN/BS-J19	74.33	25.80	24.90	6.70
VN/BS-J20	54.33	30.33	28.00	14.80
VN/BS-J21	43.67	49.80	45.33	22.00
VN/BS-J22	60.00	52.00	48.20	25.50
VN/BS-J23	125.00	55.00	50.60	32.00
VN/BS-J24	72.67	70.90	65.40	36.40
VN/BS-J25	39.33	58.50	54.60	25.00
VN/BS-J26	155.00	65.00	67.50	42.00
VN/BS-J27	13.00	35.60	34.70	15.50
VN/BS-J28	94.33	70.20	68.50	27.70
VN/BS-J29	57.00	69.50	65.20	21.83
VN/BS-J30	33.00	25.00	27.50	11.20
VN/BS-J31	336.67	20.80	19.50	3.50
VN/BS-J32	112.67	32.50	30.80	8.50
VN/BS-J33	5.00	80.00	78.50	45.00
VN/BS-J34	113.33	50.50	48.30	17.20
VN/BS-J35	32 67	48.30	47.50	20.00
VN/BS-J36	105.00	62.80	60.50	30.20
VN/BS-J37	38.00	70.30	65.20	25.30
VN/BS-J38	45.00	40.80	38.70	15.30
VN/BS-J39	65.00	52.90	50.70	20.90
VN/BS-J40	125.00	69.70	63.70	57.80
VN/BS-J41	4.64	0.85	0.50	0.98
VN/BS-J42	13.07	2.40	1.42	2.78
VN/BS-J43	10.30	3.02	1.77	6.84
Sem±	4.64	0.85	0.50	0.98
CD at 5%	13.07	2.40	1.42	2.78
CV%	10.30	3.02	1.77	6.84

* Bunching type; ** Early type; *** Precocious and prolific bearer; A High bearing, early type fruiting in bunch; B Late maturing type

1977) and wider adaptability, its dense plant population has been observed in undisturbed lands, pasture lands and on the bunds of open depressions. High population of tamarind has been observed at Guji Semal village near ponds on bund. It is general believe of the people of the region, that only fallen fruits of tamarind should be used. Three promising genotypes of tamarind have been identified on the basis of fruit character and bearing performance.

Biodiversity of Chiroji

Chiroji (*Buchanania langen*) is native to Central India and Bundel Khand region (Gupta *et al.*, 1996). During the exploration, scattered to medium dense distribution on the marginal land particularly in community places,

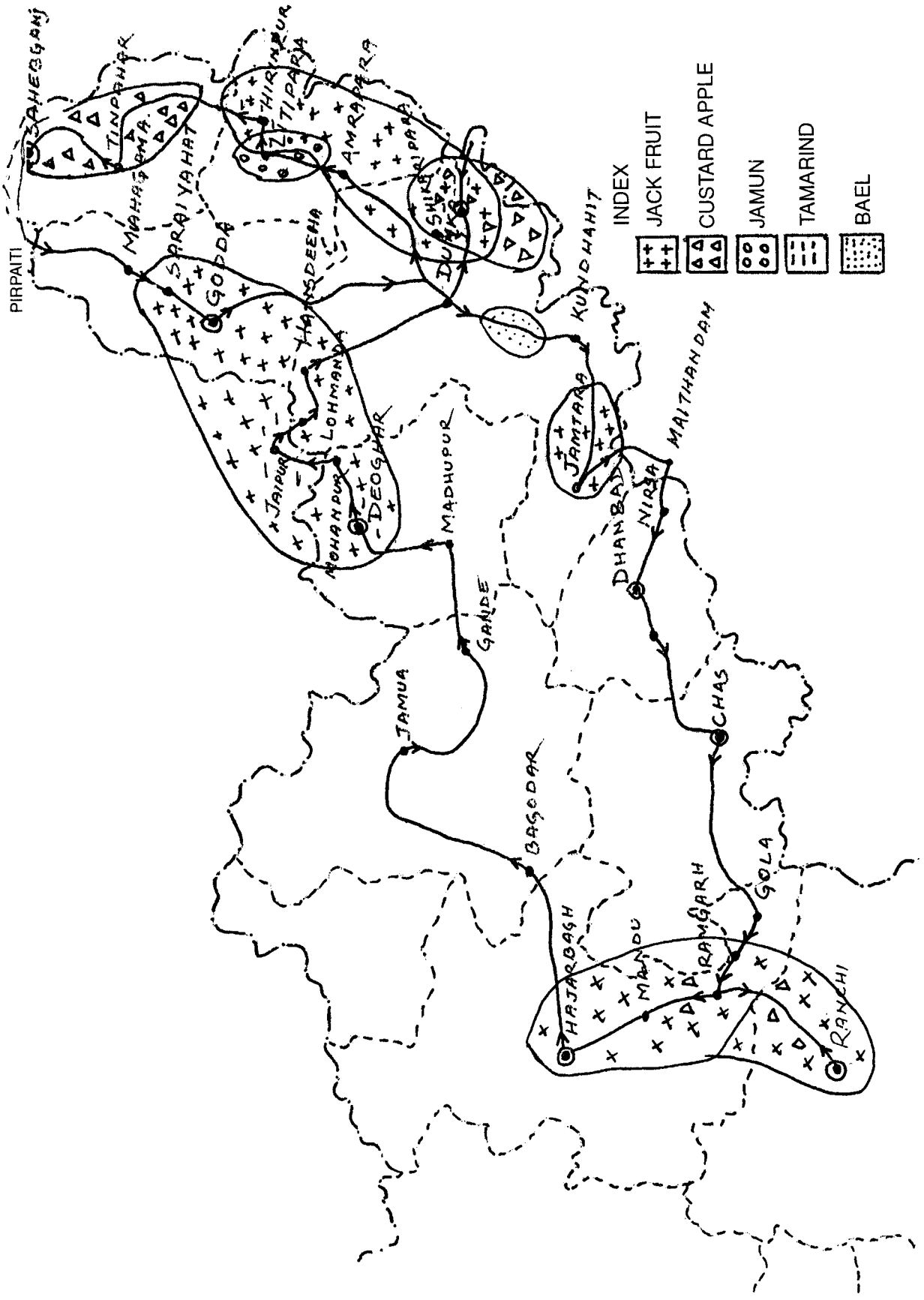

Fig. 1. Diversity of Under Utilized Fruit Crops in Santhal Pargana

undisturbed hill shopes and forest area was observed. It is one of the minor indigenous fruit that grows in natural habitat particularly in tropical region in specific pocket and possesses narrow genetic base for its fruits and tree morphological characteristics. It is one of the dry nut, known for its high nutritive value and used in garnishing sweets and dishes.

Distribution of Genetic Diversity and Collection Priority

The genetic diversity as a result of long history of cultivation, continuous sexual propagation and development of superior genotypes through conscious and unconscious selection by the farmers is eroding fast due to shrinkage of resources, deforestation, urbanisation and introduction of new crops and improved varieties. The genetic diversity of some of the fruits of the Santhal Parganas of Jharkhand, their distribution and collection priority has been listed in Table 3.

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Table 3. Fruit characters of jack fruit genotypes from Santhal Parganas

Crop/species	Diversity status	Diversity distribution	Erosion status	Collection
Mango	Rich	Wide spread	Medium	High
Jack fruit	Rich	Wide spread	High	High
Custard apple	Medium	Wide spread	Medium	Medium
Bael	Rich	Sporadic	Medium	High
Aonla	Medium	Wide spread	Medium	High
Guava	Medium	Medium	Fast	High
Jamun	Medium	Sporadic	Medium	High
Tamarind	Rich	Sporadic	High	High
Banana	Localised	Medium	Low	Medium
Chiroji	Localised	Less	Fast	High
Barhal	Localised	Wide spread	High	High
Wild dates	Rich	Medium	Medium	Medium
Wild ber	Rich	Wide spread	Medium	Medium

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SHORT COMMUNICATION

In Vitro Conservation of *Gentiana kurroo* Royle: An Indigenous Threatened Medicinal Plant

Neelam Sharma
Tissue Culture and Cryopreservation Unit, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012

In vitro conservation of shoot cultures of *Gentiana kurroo* Royle, a threatened medicinal plant, was achieved. On a standard shoot culture medium, cultures could be successfully maintained for seven months at 25°C by replacing cotton plugs with polypropylene caps as enclosures for culture tubes. Low temperature storage at 4°C, successfully extended shelf life of cultures for over 30 months. The cultures grew normally on multiplication medium.

Key Words: *Gentiana kurroo*, *In Vitro* Conservation, Low Temperature Storage, Threatened Plant

Conservation of biodiversity of medicinal plants is a shared commitment at global as well as regional level. A holistic approach to conservation is to amalgamate both *in situ* and *ex situ* methods and new emerging technologies to ensure sustained utilization. *Gentiana kurroo* Royle (Indian Gentian), commonly known as 'Karu' or 'Kutki' has been used in the indigenous system of medicine for a long time (Kirtikar and Basu, 1975, Chandel *et al.*, 1996). Dried rhizomes are not only used as a substitute for true gentian but also for treating syphilis and leucoderma.

Though a perennial herb, plants are still gathered from the natural stands by uprooting its roots and rhizome and have thus become threatened due to over exploitation (Gupta, 1988). Realizing the threat of extinction, coupled with limited documented information regarding mode of propagation and storage potential of propagules, storage of germplasm in the form of *in vitro* cultures appears to be the most promising option to ensure safe conservation of endangered plants especially those, such as *Gentiana,* in which the roots or rhizome contain the active compound.

Tissue culture techniques are invaluable to complement other conservation strategies particularly for vegetatively propagated and threatened medicinal plants. The major advantages of *in vitro* techniques are rapid multiplication and storage of relatively large number of propagules in small space, away from natural vagaries. *In vitro* multiplication of *G. kurroo* (Sharma *et al.*, 1993) and four other *Gentiana* species, namely *G. lutea, G. cruciata, G. purpurea* and *G. acaulis* (Momcilovic *et al.*, 1997) have been reported. The present paper demonstrates the feasibility of successful storage of shoot cultures of *G. kurroo* at low temperature.

Cultures of plant material obtained from All India Co-ordinated Research Project on Medicinal and Aromatic Plants Centre located at Solan, H.P., India, were initiated and multiplied using the procedure of Sharma *et al.*, (1993). Shoots were proliferated on Murashige and Skoog's (1962) medium (MS) containing 8.9 μm 6-benzyladenine (BA) and 1.1 μM 1-naphthaleneacetic acid (NAA) and 100 mg/l streptomycin. After 8 subcultures the proliferated shoots provided sufficient material for storage experiments.

Single node explants were transferred to glass tubes (25x150 mm) containing 15 ml of culture medium solidified with 0.8% agar. The tubes were closed with either cotton plugs or polypropylene caps. All the cultures were incubated at culture room conditions (Sharma *et al.*, 1993) for 2 weeks to detect and eliminate contamination. Healthy cultures were then transferred to cold module at 4° C in dark. Twenty-four replicates were used and the experiment was replicated twice. Survival of cultures was assessed by visual examination and by the ability of explant to resume growth on fresh medium.

Nodal cultures on multiplication medium produced 3-5 shoots/culture in 6 weeks from culture. High mortality was observed in cultures maintained at 25° C and covered with cotton plugs after 6 months mainly due to dessication and nutrient depletion. In contrast, cultures covered with polypropylene caps remained green and viable (100%) till 9 months. Thus, polypropylene caps were better than cotton plugs for storage of cultures. A similar response has been earlier reported for another threatened medicinally important taxa, *Rauvolfia serpentina* (Sharma and Chandel, 1992).

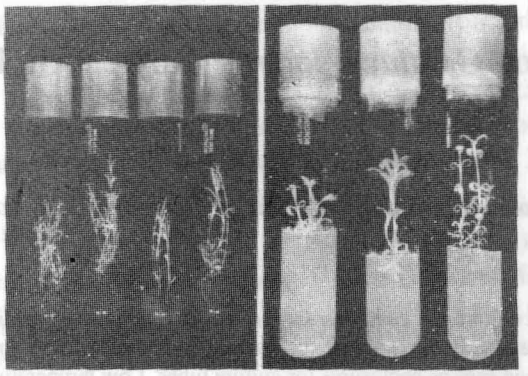

Fig. 1. In vitro *conservation of* Gentiana kurroo *(1A) Shoot cultures in vitro conserved for 30 months at 4° C; (1B) Revival of growth after 30 months of storage. Four-week-old cultures with multiple shoots*

Low temperature incubation was effective in reducing growth rate and enhancing subculture interval. Cultures remained healthy and continued to grow in dark, though slowly. Interestingly, when the shoots started senescing, new axillary sprouts were observed even after 20 months of storage. More than 50% cultures were viable and green up to 24 months. At the termination of experiment, over 40% cultures were green and viable, but only 33% were healthy after 30 months (Fig.1A). Even after this period, an average of six explants could be obtained from each surviving culture and all explants grew. Death of cultures at 4°C was probably due to depletion of nutrients and partly due to contamination. Cultures transferred to fresh medium for rejuvenation and maintained under standard growth conditions resumed normal growth and continued to produce multiple shoots (Fig. 1B).

The shoot cultures of this species have been maintained for 7 years through periodic subculturing at 25° C and 4°C, so far. The results obtained in the present investigations compare well with earlier published reports on medicinally important threatened plant species (Arora and Bhojwani 1989, Sharma and Chandel, 1992, 1996).

In vitro techniques are now successfully and routinely applied to a range of threatened species for multiplication (Chandel *et al.,* 1996). There is paucity of information regarding application of *in vitro* techniques for conservation and optimal methods still need to be developed for threatened medicinal plants which have received little scientific attention.

The present work demonstrates the feasibility of conserving threatened germplasm of *Gentiana* using *in vitro* techniques. Both short- and medium-term conservation has been achieved by incubating the cultures under normal/low temperature.

Acknowledgements

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SHORT COMMUNICATION

Heterosis and Combining Ability in Ridge Gourd

Rehana Niyaria* and MK Bhalala

Department of Plant Breeding and Genetics, B.A. College of Agriculture, GAU, Anand Campus, Anand-388110, Gujarat

** Present Address: Department of Plant Breeding and Genetics, RCA, Udaipur-313001, Rajasthan*

Key Words: Combining Ability, Heterosis, Ridge Gourd

In India, the per capita availability of vegetables falls short of the recommended requirement. Ridge gourd is a commercially important cucurbit. However, concerted efforts towards its improvement and developing new varieties are lacking and only a few varieties have been developed. Thus, it necessitates, development of high yielding, better quality varieties through efficient breeding programs. The success of any breeding procedure is determined by useful gene combinations organized in the form of high combining inbreds and heterosis in their crosses. Thus, present study was undertaken to fulfill the above objective.

The material for experimentation comprised 9 parents (PRG6, PRG7, CWRG2, Pusa Nasdar, JRG2, JRG5, JRG7, Anand 1, CO 1) and their 36 half diallel crosses of ridge gourd. These 45 genotypes were sown in Randomized Block Design with 3 replications in 1.5 x 0.5 m geometry. Each plot consisted of a single row of 10 plants. The progeny means were used for statistical analysis. Heterosis was calculated as the percentage of F_1 performance in the favorable direction of its better parent, as suggested by Hayes *et al.*, (1955) as well as standard check for each cross, while Combining ability analysis was done using Method II, Model I of Griffing (1956).

The range of heterosis over better parent and standard check for various characters is given in Table 1.

For fruit yield/plant, 6 and 22 hybrid exhibited significant positive heterosis over the better parent and standard check, respectively. Among the 6 crosses, PRG 7xCHRG 2 recorded highest percentage of heterosis for fruit yield and significant heterosis for total soluble sugars. Cross PRG 7xJRCI 5 showed significant heterosis for fruits per plant. Other than yield, heterosis was found to be significant for seeds per fruits in positive direction in cross PRG 7xPusa Nasdar, PRG 7xJRG 2 and PRG 6xPusa Nasdar. Hybrid PRG 7xAnand 1 manifested significant desirable heterosis for fruits per plant.

Table 1. Range of heterosis in 36 F_1's of ridge gourd

Characters	Range of herterosis % over	
	Better Parent	Standard Check
Days to opening of first female flower	-11.49 to 12.20	-22.69 to 13.99
Days to first picking	-11.76 to 17.97	-6.25 to 20.12
Primary branches/plant	-48.60 to 40.17	-24.60 to 57.36
Main vine length (m)	-44.97 to 54.18	1.83 to 75.49
Fruit weight (g)	-38.30 to 30.09	-35.35 to 13.70
Fruit length (cm)	-45.10 to 4.67	-45.00 to 9.49
Fruit/plant	-45.40 to 55.42	-31.53 to 154.63
Fruit yield/plant (kg)	-46.05 to 67.88	-18.11 to 121.50
Total soluble sugars (g/100g)	-63.00 to 46.45	-7.68 to 314.49

The best three crosses as per economic heterosis are discussed ahead. Cross PRG 7xAnand 1 recorded highest heterosis for fruit yield and significant heterosis for days to opening of first female flower and fruits per plant. Whereas, hybrid PRG 7xJRG 2 manifested significant heterosis for days to opening of first female flower, fruits per plant and total soluble sugars. Cross PRG 7xJRG 5 displayed significant heterosis for main vine length and fruits per plant.

All these crosses showing desirable heterosis for fruit yield also exhibited for at least one or more yield contributing characters. Thus, total yield could be the result of combinational heterosis (Ramanujam *et al.*, 1964; Das and Rai, 1972). Similar observations were made by Jamsandekar (1982) and Kadam *et al.*, (1995) in ridge gourd. The 6 crosses exhibited significant heterobeltosis for fruit yield/plant also expressed significant heterosis over standard check.

Therefore, hybrids PRG 7xJRG 2, PRG 7xJRG 5 and PRG 7xAnand 1 can be commercially cultivated on the basis of fruit yield and related characters.

The mean squares due to gca and sea suggested involvement of both additive and non-additive gene action for expression of all the characters. Therefore, improvement in this crop could be achieved by few cycles of recurrent selection.

None of the parents showed good gca effects for all the characters. The best three parents possessing significant gca effect for fruit yield/plant were Anand 1, JRG 2, and PRG 7. The other characters for which they

showed high gca are given below:

Parents	Characters having high gca
Anand 1	Primary branches per plant, main vine length, fruit length, fruit length, fruits per plant, total soluble sugars.
JRG 2	Main vine length, fruit length, fruits per plant, total soluble sugars.
PRG 7	Days to first picking, days to opening to first female flower, fruits per plant.

The top three cross combinations selected on the basis of *per se* performance have been presented in the Table 2. Cross PRG 7xAnand 1 appeared best as this exhibited significant positive sca effect and high *per se* performance for fruit yield/plant, fruits/plant and was early. At least one of the parent was a high general combiner for each of the above mentioned characters. Cross PRG 7xJRG 5 as well as PKG 7xAnand 1 showed sca effects in desirable direction though the parents

Table 2. Top three cross combinations based on *per se* performance

Characters	Cross combination	*per se* performance	sca effect	gca effects
Days to opening of first female flower	PRG6 x JRG5	31.33	-5.58	H x L
	PRG x PRG7	31.60	-1.09	H x H
	PRG 6 x CO1	31.40	-109.	H x A
Days to first picking:	PRG6 x PRG 7	40	0.33	H x H
	PRG7 x CHRG2	40	-3.09	H x A
	PRG7 x JRG5	40	-5.30	H x L
Primary branches/plant	RG7 x ANAND1	6.40	0.43	H x H
	PN x JRG7	6.07	0.47	A x H
	JRG2 x JRG7	6.00	0.59	A x H
Main Vine length	JRG2 x ANAND1	8.32	1.19	H x H
	JARG5 x ANAND1	7.99	1.08	A x H
	ANAND 1 x CO1	7.88	0.64	H x H
Fruit weight	JRG7 x ANAND1	238	1.63	H x H
	JRG2 x JRG5	218	-1.32	A x H
	JRG5 x CO1	216	-0.15	H x A
Fruit length	JRG5 x JRG7	38.23	3.45	H x H
	JRG7 x ANAND1	36.76	2.48	H x H
	P.N x JRG5	36.41	1.65	H x H
Fruits/plant	PRG6 x ANAND1	11.03	1.82	H x H
	PRG7 x JRG2	1.65	0.36	H x H
	PRG7 x JRG5	1.64	0.37	H x A
Total soluble sugars	ANAND1 x CO1	7.73	2.19	H x H
	JRG2 x ANAND1	6.58	1.32	H x H
	JRG5 x CO1	6.55	1.96	A x H

involved in these crosses were not all high general combiners. This may be due to intra-allelic interactions. The same was observed by Shaha *et al.*, (1999) and Kadam (1989) in ridge gourd. Undesirable sca effects were observed in cross JRG 2xJRG 5 for fruit weight and PRG 6xPRG 7 for early picking, though the parents were high or average general combiners. The reason could be failure of alleles to co-adapt. Thus from the experiment, PRG 7xAnand 1 and PRG 7xJRG 5 were found desirable for yield as well as earliness and could be exploited in a breeding program to develop a variety.

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SHORT COMMUNICATION

Collection and Evaluation of Wild Betelvine Germplasm from Andaman Islands

TVRS Sharma, A Sujatha Nair, MA Suryanarayana and PV Sreekumar*
Central Agricultural Research Institute, Port Blair-744101, Andaman and Nicobar Islands
** Botanical Survey of India, Port Blair-744102, Andaman and Nicobar Islands*

Key Words: Betel, Evaluation, Wild Types

Betel vine (*Piper betle* L.) is a prominent cash crop cultivated for its leaves, with a strong aromatic flavour, which is used for masticatory purposes in combination with lime, arecanut and so on. It finds a significant place in our socio-cultural life and religious ceremonies, besides having a number of medicinal properties.

In the earlier literature available, there is no report of betel vine occurring in the wild in Indian mainland (Ellis and Vishnoi, 1989). The Andaman and Nicobar group of islands in the Bay of Bengal is situated between 6°-14° N latitudes and 92°-94° E longitudes with warm and humid tropical climate. The average annual rainfall is 3800 mm with 80% relative humidity and the temperature ranging from 22 to 32°C. The first report of betel vine growing in wild condition was by Kurz (1876) who observed the availability of both cultivated and wild plants in the beach forests of Nicobar islands. Betel vine in the wild state was found to occur in the inland forests also, Kloss (1903) observed wild betel vine also known as 'Sirah leaf', occurring in the wild from Northern Andaman islands to Southern most Great Nicobar. Sreekumar and Ellis (1990) reported six wild relatives of betel vine from Great Nicobar which are used by the local tribes 'Shompens'. They also opined the Great Nicobar might be the center of origin of this economically important plant species, as lot of diversity is prevalent and no other report of this species in the wild has been given in any botanical work. Sreekumar *et al.*, (1996) observed that *Piper betle* L. is one of the 250 economically important vascular plant growing wild in the islands and is being used by the inhabitants.

The collection and conservation of these wild species assume significance. Efforts are being made by the Central Agricultural Research Institute, Port Blair to collect and increase the betel vine germplasm. Eight

Table 1. A brief description of the germplasm collection

Description	CARI-B1	CARI-B2	CARI-B3	CARI-B4	CARI-B5	CARI-B6	CARI-B7-1	CARI-B8
1. Source (S/Andaman)	Choudhari (S/Andaman)	Choudhari	Rutland	Rutland	Rutland	Protherapur (S/Andaman)	S/Andaman	S/Andaman
2. Natural Hahitat	Near streams	Near streams	Near streams and in shade of large trees	Shade loving	Near streams dark shady areas	Forest	Near streams	Near streams
3. Leaf size								
Length (cm)	17.69	10.59	5.0	9.67	14.33	8.18	12.22	11.86
Breadth (cm)	9.33	5.68	4.0	6.4	4.67	6.38	9.3	9.43
Colour	Green	Green	Green	Green	Green	Green	Dark green	Dark green
Venation	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Petiole length (cm)	6.05	6.0	3.0	5.0	5.0	3.06	5.24	6.8
4. Internodal Length(cm)	12.87	7.0	4.03	11.33	10.0	3.02	7.94	11.7
5. Stem colour	Green	Green	Pink pigment	Green	Green	Greyish green	Green	Green
6. Prefernce for chewing	Very much preferred by the locals	Not much preferred	Not used for chewing	Not Much preferred	Very much preferred for chewing	Moderate	Prefered for chewing	Most preferred for chewing
7. Other characters	Plants are vigorous but cannot withstand sunshine	Plants are less vigorous with small thick leaves and thin stem	Plants are rare. Absolutely no damage by any pests	-	Long and narrow leaves	Plant is short in stature and not vigorous	Plant is vigorous	Leaf is acute and pointed disease and pest incidence is nil.

genotypes of wild germplasm of betel vine collected so far from North and South Andamans are being evaluated for desirable traits. Table 1 presents the evaluation date of eight wild genotypes of betel vine. Among the eight wild collections described, CARI-B7-1 is found to have potential for cultivation purpose as well as for use in crop improvement work. This collection is found to be free of pest infestation and disease incidence and is also preferred very much by the local people for chewing. The fresh weight of 100 leaves is 219.30 and it keeps well 23 days under controlled storage conditions (leaves kept in perforated polybags). Multiplication of this promising variety of betel vine is progressively going on at CARI, so as to put it into use for future crop improvement programme.

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Plant Germplasm Registration Notice

The Germplasm Registration Committee of ICAR in its seventh meeting held on 29th January 2001 approved the registration of following 18 germplasm lines/genetic stocks of the 51 proposals received.

KBRL 10 (Reg. No. INGR 01001) Wheat

KBRL 10, Reg. No. INGR 01001 is a Karnal Bunt [*Neovossia indica* (Mitra) Mundukar] resistant line of bread wheat (*Triticum aestivum* L.) developed at Punjab Agricultural University, Ludhiana. It is developed by crossing Karnal Bunt resistant stocks, HP 1531 x HD 29, followed by backcrossing the F_1 generation with HP1531. Screening for individual plant progenies from F_2 resulted in the selection of Karnal Bunt-free plants. Further, screening and selection for the disease free plants continued in subsequent generations till homozygosity was attained. The final selections were screened under artificial inoculation, both against mixtures and individual isolates of *Neovossia indica* to confirm the resistance potential. These efforts have resulted in pyramiding the genes conferring Karnal Bunt resistance.

Indu Sharma, GS Nanda and Karam Chand
Punjab Agricultural University, Ludhiana-141004

KBRL 13 (Reg. No. INGR 01002) Wheat

KBRL 13, Reg. No. INGR 01002 is another Karnal Bunt [*Neovossia indica* (Mitra) Mundukar] resistant line of bread wheat (*Triticum aestivum* L.) developed at Punjab Agricultural University, Ludhiana. KBRL 13 is developed by crossing Karnal Bunt resistant stocks, W 485 x HD 29, followed by backcrossing of F_1 plants with HD 29. Individual plant progenies from F_2 were screened. Selection of Karnal Bunt-free plants was made in subsequent generations until uniformity. The final selections were screened under artificial inoculation against both individual and mixtures of isolates of *Neovossia indica* for three years to confirm the resistance potential. These efforts have resulted in pyramiding the genes conferring Karnal Bunt resistance.

Indu Sharma, GS Nanda and Karam Chand
Punjab Agricultural University, Ludhiana-141004

KBRL 22 (Reg. No. INGR 01003) Wheat

KBRL 22, Reg. No. INGR 01003, is a Karnal Bunt [*Neovossia indica* (Mitra) Mundukar] resistant line of bread wheat (*Triticum aestivum* L.) developed at Punjab Agricultural University, Ludhiana. KBRL 22 is developed by crossing Karnal Bunt resistant stocks, W 485 x HD 29 followed by backcrossing of F_1 plants with W 485. Screening of individual plant progenies from F_2 and in subsequent generations against Karnal Bunt resulted in identification of disease-free plants and isolation of a uniform line. The identified material was finally screened against artificial inoculation of both mixtures and individual isolates of *Neovossia indica* for three years to ascertain the resistance potential. This has resulted in the production of a Karnal Bunt resistance source by pyramiding the genes conferring Karnal Bunt resistance.

Indu Sharma, GS Nanda and Karam Chand
Punjab Agricultural University, Ludhiana-141004

WH 595 (Reg. No. INGR 01004) Wheat

WH 595, Reg. No. INGR 01004 is a leaf rust (*Puccinia recondita* Rob. ex Desm.) and stripe rust [*Puccinia glumarum* (Schmidt) Erikes. & Henn.] resistant genetic stock of bread wheat (*Triticum aestivum* L.) with excellent bread making quality and high protein content (13.95%). It was developed at Chaudhary Charan Singh, Haryana Agricultural University, Hisar, through single plant selection in PRL 6045/NAC 76. INGR 01004 has yield comparable to the available released cultivar, in addition to resistant potential.

Mohd Yunus, AS Redhu, LS Panwar, RB Srivastava, MS Beniwal and AK Sharma
Chaudhary Charan Singh Haryana Agricultural University, Hisar-125004

WH 712 (Reg. No. INGR 01005) Wheat

WH 712, Reg. No. INGR 01005 is a genetic stock of bread wheat (*Triticum aestivum* L.) with high sedimentation value (63.6%) among the varieties tested in Advance Variety Trial in North West Plain Zone. It has 12.4% protein content with 5.7 grain appearance score on 9 point scale and also possess 5+10 sub-units of glutenin. It is developed at Chaudhary Charan Singh, Haryana Agricultural University, Hisar through pedigree selection from TRAP#1 //BBW/PFAU.

Mohd Yunus, RB Yadav AS Redhu, IS Panwar, Sashi Madan and B[illegible] Mishra
Chaudhary Charan Singh Haryana Agricultural University, Hisar-125004

BH 561 (Reg. No. INGR 01006) Barley

BH 561, Reg. No. INGR 01006 is multi-pistillate genotype of barley (*Hordeum vulgare* L.) with four ovaries. It was identified at Chaudhary Charan Singh, Haryana Agricultural University, Hisar in 1993 as a natural variant in F_2 population of cross, BG 105 x K 125. The plant type is normal, but without stamens in the open florets

of the spike. Genetically a single recessive gene governs this character. However, only one seed develops in each floret, which is bold, hulled with good germination percentage.

Dalvir Singh and SK Sethi
Chaudhary Charan Singh Haryana Agricultural University, Hisar-125004

BH 562 (Reg. No. INGR 01007) Barley Germplasm

BH 562, Reg. No. INGR 01007, is a 6-rowed genic male sterile line of barley (*Hordeum vulgar* L.) developed at Chaudhary Charan Singh, Haryana Agricultural University, Hisar. It was produced by the treatment of RD 2508 with gamma ray irradiation ^{60}Co (30 kR). The male sterility in BH562 is controlled by single recessive gene. INGR 01007 can be a good source for developing genotypes suitable for rainfed conditions using male sterile facilitated recurrent selection.

Dalvir Singh, VS Malik and Krishan Kumar
Chaudhary Charan Singh Haryana Agricultural University, Hisar-125004

BH 563 (Reg. No. INGR 01008) Barley Germplasm

BH 563, Reg. No. INGR 01008 is a 2-rowed genic male sterile line of barley (*Hordeum vulgare* L.) developed at Chaudhary Charan Singh, Haryana Agricultural University, Hisar. This line was identified in Alfa 93 population treated with sodium azide (NaN_3) of a concentration of 10^{-2} M. The male sterility is controlled by single recessive gene. BH 563 can be used as a source for developing genotypes suitable for malting quality through male sterile facilitated recurrent selection.

Dalvir Singh, VS Malik and Krishan Kumar
Chaudhary Charan Singh Haryana Agricultural University, Hisar-125 004

DPC 9 (Reg. No. INGR 01009) Castor

DPC 9, Reg. No. INGR 01009 is a non-revertant, early stable pistillate genotype of castor (*Ricinus communis* L.) developed at Directorate of Oilseeds Research (DOR), Hyderabad with green stem, zero bloom and spiny capsules. It was developed through hybridisation and pedigree selection method in crosses of VP-1 x (Bhagya x CO-1) for pistillate behaviour. VP-1 was used as source for pistillate character. In addition, INGR 01009 gave the highest total quantity of seed (270.4 g/plant) in its growth period, and also had the highest oil content (53.4%) compared to other pistillate lines evaluated. The development of pistillate type under different genetic background is essential for hybrid breeding in castor.

SK Chakarbarthy, M Ramachandran, C Hanumantha Rao and C Lavanya
Directorate of Oilseeds Research, Hyderabad-500030

LRES 17 (Reg. No. INGR 01010) Castor

LRES 17, Reg. No. INGR 01010, is an S type pistillate genotype of castor (*Ricinus communis* L.), developed at Directorate of Oilseeds Research (DOR), Hyderabad, with green stem, triple bloom, dwarf plant type and spiny capsules. It is developed by pedigree selection method in a cross between VP-1 x HC 8, for pistillate behaviour.

SK Chakarbarthy, M Ramachandran, C Hanumantha Rao and C Lavanya
Directorate of Oilseeds Research, Hyderabad-500030

EX/A680-16 (Reg. No. INGR 01011) Potato Germplasm

EX/A680-16, Reg. No. INGR 01011 is short day adapted potato (*Solanum tuberosum* ssp. *andigena* L.) with resistance to early blight caused by *Alternaria solani* and late blight caused by *Phytopthora infestans* (Mont) de Bary. It was developed by clonal selection at Central Potato Research Institute, Shimla. The clone was identified in population of NO 502-14 x Bulk Pollen. It is an exceptionally good combiner for most characters, including tuber yield and its components, and resistance for early and late blight.

PC Gaur, Jai Gopal, SK Pandey and MS Rana
Central Potato Research Institute, Shimla-171001

Hisar Methi 346 (Reg. No. INGR 01012) Fenugreek

Hisar Methi-346, Reg. No. INGR 01012 is a downy mildew (*Pernospora trigonellae* Gaum.) resistant germplasm of fenugreek (*Trigonella foenum-graecum* L.). It has quick germination, faster initial growth, long pod, bold seed with green tan seed coat colour, and light green narrow leaves. It is a induced mutant, identified in IL-355-1 germplasm line, maintained as pure line at Chaudhary Charan Singh, Haryana Agricultural University, Hisar.

PS Partap, MK Rana and Rakesh Mehra
Chaudhary Charan Singh Haryana Agricultural University, Hisar-125004

Hisar Methi 350 (Reg. No. INGR 01013) Fenugreek

Hisar Methi 350, Reg. No. 01013 is a powdery mildew (*Erysiphe polygoni* DC.) resistant and downy mildew (*Pernospora trigonellae* Gaum.) tolerant germplasm of fenugreek (*Trigonella foenum-graecum* L.). The plant is tall erect, multi branched with whitish green stems and dark green narrow leaves. It was identified as a single plant selection from PLME 46-1 at Chaudhary Charan Singh, Haryana Agricultural University, Hisar.

PS Partap, MK Rana and Rakesh Mehra
Chaudhary Charan Singh Haryana Agricultural University, Hisar-125004

***Trombay* Sesbania rostrata-*1 (TSR-1) (Reg. No. INGR 01014)* Sesbania**

TSR-1 Sesbania (*Sesbania rostrata*), Reg. No. INGR 01014 was identified, at Nuclear Agriculture and Biotechnology Division of Babha Atomic Research Centre, in M_2 generation of seeds treated with gamma rays. The parent *Sesbania rostrata* is sensitive to photoperiod and flowers after 60 days of vegetative growth. The mutant TSR-1, by virtue of its photoperiod insensitivity has longer vegetative phase compared to parent in winter and normal vegetative phase in other seasons. TSR-1 therefore, has potential for producing sufficient phyto-mass regardless of growing time.

DC Joshua, Saradha Ramani and MS Shaikh
Babha Atomic Research Centre, Bombay-400085

KPL 44 (Reg. No. INGR 01015) Pigeonpea

KPL 44, Reg. No. INGR 01015, a long duration pigeonpea (*Cajanas cajan* Linn.), is a source of multiple disease resistance. It has resistance to *Fusarium udum* Bult., sterility mosaic and *Phytopthora drechsleri*. It is developed by pedigree selection from a segregating material at Indian Institute of Pulses Research, Kanpur.

DP Srivastava, Vishwadhar, RG Chaudhary and Naimuddin
Indian Institute of Pulses Research, Kanpur-208024

Safeda Rohtak (Reg. No. INGR 01016) Ber

Safeda Rohtak, Reg. No. INGR 01016 is a powdery mildew (*Oidium erysiphoides f. ziziphi*) resistant line of ber (*Zizyphus mauritiana* Lam.). It is a selection from local orchards of Rohtak (Haryana) and has been established at Hisar. The tree is a spreading type with lesser thorns. Tree height is 5.9 m, trunk girth 1.4 m with wide spread. Fruits are of small size having pointed apex and average weight 15.2 g, total soluble sugars 17 %, acidity 0.22 %, ascorbic acid 108.00 mg/100g pulp. Fruit yield is 100-120 Kg per tree.

NR Godhara, AK Gupta, SK Bhatia, RS Saini,
PC Gupta and RL Madan
Chaudhary Charan Singh Haryana Agricultural University,
Hisar-125004

B.S.75-1 (Reg. No. INGR 01017) Ber

B.S.75-1, Reg. No. INGR 01017 is a fruit fly resistant line of ber (*Zizyphus mauritiana* Lam.) with sweet flavoured fruit. It is a selection made from local orchards of Bawal, Rewari, Haryana and established at Chaudhary Charan Singh, Haryana Agricultural University Hisar. The tree is of medium size, having height of 5.80 m, trunk girth 0.93 m with medium spread. Fruits are dark green before ripening. Average fruit weight is 15.5 g, total soluble sugars 19 %, acidity 0.28 %, Ascorbic acid 106 mg/100 gm pulp. Fruit yield is 150 kg per tree.

NR Godhara, AK Gupta, SK Bhatia, RS Saini,
PC Gupta and RL Madan
Chaudhary Charan Singh Haryana Agricultural University
Hisar-125004

BS75-3 (Reg. No. INGR 01018) Ber

B.S.75-3, Reg. No. INGR 01018 is a fruit fly resistant line of ber (*Zizyphus mauritiana* Lam.) with sweet flavoured fruit. It is a selection from local orchids of Bawal, Rewari, Haryana, established at Chaudhary Charan Singh Haryana Agricultural University Hisar. The tree is of medium size, spreading type having height 5.20 m, trunk girth 1.37 m and medium spread. The leaf is round with glossy upper surface. Average weight of the fruit is 14.2 gm, total soluble sugars 17 %, acidity 0.30 %, ascorbic acid 100 mg/100 g pulp. Fruit yield is 150 kg/tree.

NR Godhara, AK Gupta, SK Bhatia, RS Saini,
PC Gupta and RL Madan
Chaudhary Charan Singh Haryana Agricultural University
Hisar-125004

Errata

Plant Germplasm Registration 1999. *Indian J. Pl. Genet. Resour.* **12**: 273-275:

Page 273, para 8, line 4 on INGR 99007, TERI-GAURAV (TERI-00-R 985) should read TERI-GAURAV (TERI-00-R 986) and Page 273, para 9, line 4 on INGR 99008, TERI-GARMIA (TERI-00-R 986) should read TERI-GARIMA (TERI-00-R 985)

Communicated by Dr AK Singh, Member Secretary, Plant Germplasm Registration Committee, NBPGR, New Delhi.

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