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Optimal use of SSRs for Establishing Genetic Relationships and Variety Identification in a Collection of Sugarcane Hybrids

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Simple sequence repeat (SSR) analysis was carried out to assess genetic diversity and to establish genetic relatedness among sugarcane hybrids in a collection of 62 genotypes. Molecular data were also used to determine the discriminating power and utility of different SSR primers for sugarcane genotype identification and to find the optimal primer combination to ensure unambiguous identification. In 62 sugarcane hybrids, a total of 107 loci were detected with 13 SSR primers. The percentage of polymorphic loci was quite high (94%), with the NKS23, mSSCIR19 and NKS34 primers revealing the highest levels of polymorphism. The dissimilarity coefficients ranged from 0.064 to 0.68 with a mean of 0.46. The majority-rule consensus tree showed eight main clusters supported by more than 50% occurrences with several subgroups with the maximum bootstrap score (100%). A total of 315 banding patterns were detected. Discrimination power analysis revealed that the efficiency of a given primer does not depend only on the number of bands or the banding pattern but also on the frequency of differences between patterns generated by the primers. The primers NKS23 and mSSCIR19 generated patterns at the same frequency (isofrequencies) and have a maximal discriminating power. PIC values close to 1 (0.719 to 0.985) demonstrated that, in spite of the low number of primers, these SSR were sufficiently polymorphic, discriminating, and informative and will be useful in sugarcane variety registration and in genetic identity tests.

Key Words: Discrimination power, Genetic Diversity, SSR, Sugarcane

Germplasm collections are important in genetic conservation and as a component of plant improvement programs by providing plant breeders with sources of useful traits. Both cultivated and wild species are, commonly maintained in germplasm banks. The screening and the evaluation of the genetic diversity in these collections is essential to sustain scientific-based actions, not only to optimize and facilitate the breeding process but also to provide management strategies to maintain high degree of variability of the breeding materials. Given the number of genotypes present in collections, it is important to unambiguously distinguish between accessions to clarify synonyms and for properly attending rights protection.

The effectiveness in exploring diversity varies with the nature of traits (morphological, genealogical records, biochemical, chromosomal or molecular characters). However, it has been suggested that the underlying genetic diversity in cultivated sugarcane is quite narrow. Most of the sugarcane varieties share common ancestors derived from early few crosses involving *Saccharum officinarum* and *S. spontaneum* in the early nobilization process. Many Indian cultivars have been introduced and used in crosses in most sugarcane breeding programs in USA. For instance many Co cultivars were ancestral parent of LCP 85-384, several generations back (Liu *et al*., 2011). Compared to other crops, sugarcane breeding programs have historically been slow to progress because of the low efficiency and technical difficulties in crossing and selection processes (Chen *et al.*, 2009) as well as the narrow genetic base from which the original material was used in founding crosses. A common aim for many sugarcane breeding programs in the world is to increase the genetic base by introducing variability from related wild species, thus, it is necessary to accurately assess the genetic variability currently available in germplasm banks. Many methods have been used to evaluate genetic variability and genetic relationships among breeding materials in this crop. Although recent studies have been based on pedigrees, this approach is often constrained by the availability of accurate and complete information. The traditional methods that combine agronomical and morphological traits are not effective for this purpose because many of these characters are influenced by

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environmental factors and, thus, do not reflect underlying diversity. Morphological markers do not allow for reliable discrimination of sugarcane accessions when they are closely related. Molecular markers, however, do permit discrimination among commercial cultivars and provide accurate estimations of their genetic relationships. In the complex polyploid aneuploid sugarcane (*Saccharum* spp.), which harbors in excess of 100 chromosomes, interpretation and analysis of data generated by amplification of simple sequence repeats (SSRs) is more difficult than it is in diploid species (Glynn *et al.,* 2009). The multiple copies of homologous chromosomes present in the sugarcane genome make SSR manual band scoring difficult. However, the hypervariability of SSRs among related organisms make them an informative and excellent choice of marker for a wide range of applications in this crop, including genotype identification and analysis of genetic diversity (Pan *et al.,* 2007; Cordeiro *et al.,* 2003). Thus, to differentiate large numbers of sugarcane materials and establish genetic relationships among them, it is important to detect those SSRs that are easily scored and have a sufficient level of polymorphism. These parameters will not only ensure a low cost of analysis but also guarantee genotype discrimination and reduce the risk of confusion of any of these genotypes with a randomly chosen accession from a larger sample of the germplasm bank (Belaj *et al.*, 2004).

Tessier *et al.,* (1999) developed the parameter *Discriminating Power* by means of which the efficiency of a given primer used alone or in combination with others can be evaluated for the identification of varieties.

The objectives of this study were as follows: (1) to assess genetic diversity among sugarcane hybrids in the sugarcane breeding collection at INTA (Argentina), (2) to establish genetic relatedness among these materials, (3) to determine the discriminating power and effectiveness of different SSR primers for sugarcane genotype identification and (4) to determine the optimal SSR primer combination to ensure unambiguous identification of a set of sugarcane genotypes.

Materials and Methods

Plant Material

Sixty two sugarcane hybrids from a breeding collection at INTA (Tucumán, Argentina) mostly derived from *S officinarum* and *S. spontaneaum* were included in this study (Table 1). These genotypes are of interest for breeding purposes in Argentina due to their adaptability to subtropical areas (short cycle and early maturity). Some of these materials are or were used as commercial varieties in Argentina. Materials investigated are listed in Table 1. From these, F97-786, F97-395 and F98-70, are suspected to be duplicates of some other varieties studied based on field morphological resemblance. TUC77-42b is a suspected duplicate of TUC77-42 collected in a different site.

DNA Extraction

Total genomic DNA was extracted from young leaves according to Doyle and Doyle, (1987).

SSR Amplification

Based on the consistency of band patterns obtained in a previous study, 13 SSR primers were chosen (Table 2). Polymerase chain reactions (PCRs) were performed with 20 µl reaction mixes consisting of 6 ng of template DNA,

Table1. Sugarcane varieties included in the genetic variability analysis and country of origin

Varieties	Country of Origin	Varieties	Country of Origin
LCP85-384	Louisiana, USA	US74-1011	Louisiana, USA
LCP86-454	Louisiana, USA	US74--1015	Louisiana, USA
LCP85-376	Louisiana, USA	US72-1289	Louisiana, USA
NCO310	Natal, South Africa	L75-33	Louisiana, USA
HoCP85-845	Louisiana, USA	TCP81-3067	Tucumán, Argentina
HoCP92-648	Louisiana, USA	TCP87-388	Tucumán, Argentina
HoCP92-645	Louisiana, USA	NA78-724	Salta, Argentina
HoCP92-624	Louisiana, USA	NA84-3471	Salta, Argentina
HoCP89-888	Louisiana, USA	NA63-90	Salta, Argentina
HoCP91-552	Louisiana, USA	NA76-128	Salta, Argentina
HoCP92-631	Louisiana, USA	NA73-2596	Salta, Argentina
HoCP91-555	Louisiana, USA	NA88-948	Salta, Argentina
HoCP88-739	Louisiana, USA	NA73-1454	Salta, Argentina
HoCP90-941	Louisiana, USA	CP48-103	Louisiana, USA
CP70-1133	Louisiana, USA	TUC67-24	Tucumán, Argentina
CP79-1380	Louisiana, USA	TUC71-7	Tucumán, Argentina
CP79-318	Louisiana, USA	TUC68-18	Tucumán, Argentina
CP65-350	Louisiana, USA	TUC67-24	Tucumán, Argentina
CP57-603	Louisiana, USA	TUC79-9	Tucumán, Argentina
CP57-614	Louisiana, USA	TUC78-39	Tucumán, Argentina
CP72-2086	Louisiana, USA	TUC72-4	Tucumán, Argentina
CP66-346	Louisiana, USA	TUC69-2	Tucumán, Argentina
CP62-258	Louisiana, USA	L91-281	Louisiana, USA
FAM81-820	Tucumán, Argentina	RA89-686	Tucumán, Argentina
FAM83-11	Tucumán, Argentina	RA87-2	Tucumán, Argentina
TUC80-7	Tucumán, Argentina	RA91-209	Tucumán, Argentina
TUC72-16	Tucumán, Argentina	RA93-154	Tucumán, Argentina
TUC74-6	Tucumán, Argentina	CP88-1834	Louisiana, USA
CP87-357	Louisiana, USA	F98-70	Tucumán, Argentina
TUC71-7	Tucumán, Argentina	F97-395	Tucumán, Argentina
TUC68-18	Tucumán, Argentina	F97-786	Tucumán, Argentina
TUCCP77-42	Tucumán, Argentina	CP65-357	Louisiana, USA
TUCCP77-42b	Tucumán, Argentina		

Table 2. SSR primers used for genotyping 62 sugarcane accessions from the INTA Sugarcane Breeding Collection (Tucumán, Argentina)

SSR	Repit Motif	Size Range (bp)	Annealing Tm (ºC)	Forward Primer sequence (5` to 3`) Reverse Primer sequence (5` to 3`)
NKS26	$(TG)_{18}$	194-164	54	GTT CTC GAC ATG GGC CTA CT CTG CAC TTT CGG TCC TTT TT
mSSCIR19	$(GA)_{23}$	130-160	48	GGT TCC AAA ATA CAC AAA CAA TCT TAT CTA CGC ACT T
NKS38	$(AG)_{15}$	92-292	55	TGA ACT CGG CAA CAG TTT TT CCC ACC AAG TCG TTC TGA AT
NKS 23	$(GA)_{18}$	113-498	54	TAA ACC CCC GAA AAA GAA CC TCC GGA GGT AGA TCC ATT TG
NKS34	$(GT)_{18}$ $(A)_{31}$	131-214	58	CGT CTT GTG GAT TGG ATT GG TGG ATT GCT CAG GTG TTT CA
mSSCIR16	$(GA)_{18}$	130-300	54	TGG GGA GGG CTG ACT AGA GGC GGT ATA TAT GCT GTG
SMC703BS	$(CA)_{12}$	186-229	62	GCC TTT CTC CAA ACC AAT TAG T GTT GTT TAT GGA ATG GTG AGG A
mSSCIR3	$(GT)_{28}$	171-187	60	AAT GCT CCC ACA CCA AAT GC GGA CTA CTC CAC AAT GAT GC
mSSCIR18	$(GA)_{23}$	170-200	52	GGG TGT TCT GTT GAG CA GAG GTA GGA GGG AGT GTT
SMC766BS	$(CA)_{20}$ $(GA)_{16}$	170-270	60	TTA CTC GGC TGG GTT TTG TTC TAA GAA TCG TTC GCT CCA GC
SMC7CUQ	$(CA)_{10}$ $(C)_4$	160-170	60	GCC AAA GCA AGG GTC ACT AGA AGC TCT ATC AGT TGA AAC CGA
mSSCIR78	$(GTT)_6$	150-310	48	TGCCTTAAC CGT GAC ATC GAGGACGAGGAGCAGAA
mSSCIR34	(GA)	130-300	56	ATCGCCTCCACTAAATAAT TTGTCTTTGCTTCCTCCTC

0.1 mM dNTPs, 0.25 mM SSR primers (forward and reverse), 1 U of GoTaq DNA Polymerase (Promega) and 1X reaction buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$). PCRs were carried out in a thermal cycler (Techne TC-412) using a program of 94ºC for 4 min and 35 cycles of 45 sec at 94ºC, 45 sec at 56ºC and 1 min at 72ºC. Each of the amplifications was repeated at least twice by independent PCR to examine the reproducibility and confirm band patterns. Amplification products were separated on 6% denaturing polyacrylamide gels using a GibcoBRL Model S2 Sequencing Gel Electrophoresis Apparatus (Life Technologies, Paisley, Scotland). Electrophoresis was carried out for 1.5 h at 60 W. At the end of electrophoresis, the gels were stained with silver nitrate according to Creste *et al.,* (2001). The resulting banding pattern was scored manually. Only consistent bands with strong intensity were considered for the analysis.

Data Analysis

Each band was treated as a unit locus, and the genotype was scored for the presence (1) or absence (0) of a band.

To measure the amount of genetic diversity, the polymorphism or rate of polymorphism was estimated as the proportion of polymorphic bands over the total number of markers per SSR primer.

Genetic dissimilarities among all possible pairs of sugarcane accessions were calculated as $d=1-s_{ij}$, where s_{ij} corresponds to the Jaccard similarity coefficient. Based on dissimilarity matrices, a hierarchical cluster analysis was performed using the UPGMA (Unweighted Pair Group Method using Arithmetic Average), and the relationships between all pairs of genotypes were visualized as a majority rule consensus tree (consensus threshold 50%). The cluster analysis was validated by bootstrap analysis with 1000 replications using FAMD software (Schluter and Harris, 2006). According to Highton, (1993) the limit of 50% was considered to indicate statistical support for the topology at a node in the bootstrap consensus tree.

To select the subset of SSR primers that produce informative profiles and to determine the discriminating power of each marker, the efficiency of a SSR primer

was evaluated using the following parameters for each assay unit (*U*) (the product of PCR amplification obtained with one set of primers) (Belaj *et al.*, 2004):

1. Number of polymorphic bands (n_p)
2. Number of non-polymorphic bands (n_{np})
3. Average number of polymorphic bands per assay unit (n_p/U)
4. Number of loci (*L*): Although SSRs are classified as co-dominant markers, given the highly complex genome of *Saccharum*, they have been treated as dominant markers, and each SSR marker represents a single locus (Cordeiro *et al.*, 2003).
5. Number of banding pattern for each SSR primer (T_p)
6. Confusion probability (C_j) on the jth assay unit: $c_j = \sum_{i=1}^{j} P_i \frac{(Npi-1)}{N-1}$, where p_i is the frequency of the ith pattern; *N*, sample size; *I*, total number of patterns generated by the primer
7. Discriminating power (D_j) of the jth assay unit: $D_j = 1 - C_j = 1 - \sum_{i=1}^{j} P_i \frac{(Npi-1)}{N-1}$
8. Limit of D_j as *N* tends toward infinity: $D_L = lim\ (D_j) = 1 - \sum_{i=1}^{j} P_i^2$
9. Effective number of patterns per assay unit: $P = \frac{1}{1-Dj}$

The optimal set of primer combinations for identification purposes was evaluated as described by Belaj *et al.*, (2004). In the set of 62 accessions, it is possible to find N(N-1)/2 different pairs; thus, the theoretical number of non-distinguishable pairs of genotypes is given by x_k= [N(N-1)/2]C_k, where C_k is the Joint confusion probability and is a product of the C_j of each primer under the independence hypothesis.

Results and Discussion

Even though SSRs are classified as co-dominant markers, their treatment as dominant markers was necessary to analyze the highly complex genome of *Saccharum*. All of the tested primers successfully amplified DNA from the Sugarcane Breeding Collection at INTA. With 13 SSR primers, 107 loci were detected in the set of 62 sugarcane varieties. The number of loci per marker ranged from 4 to 13 (Table 3). Marker size ranged between 110 to 310 bp. Primers that generated substantial polymorphism among the sugarcane accessions were detected. Of 107 fragments scored, 100 were polymorphic (94%), and the seven remaining showed monomorphic patterns (4.9%). The primers NKS23, mSSCIR19 and NKS34 generated the highest levels of polymorphism with 13, 12 and 12 polymorphic fragments, respectively. Earlier studies reported similar levels of polymorphism (Aitken *et al.*, 2006; Creste *et al.*, 2010; Singh *et al.*, 2010; Choperena *et al.*, 2009). These authors have estimated the polymorphism by AFLP or SSR markers. Although these markers have different distributions along nuclear DNA, the *P* values do not significantly differ. The high percentage of polymorphism can be explained because of the complex polyploid aneuploid in sugarcane. Working with EST-SSR, Ukoskit *et al.*, (2012) described high values of percentage of polymorphism (93.7%). They suggested that different chromosome numbers could

Table 3. Comparison of informativeness obtained from a set of 12 SSR primers in 62 sugarcane genotypes. Number of polymorphic bands (n_p), Number of non-polymorphic bands (n_{np}), Number of loci (*L*), Number of banding pattern for each SSR primer (T_p), Confusion probability (C_j), Effective number of patterns per assay unit (*P*). Primer discriminating power calculated from the sample of 62 sugarcane accessions (D_j) and estimated as *N* tends toward infinity (D_L). Number of varieties unique identified (*VI*)

SSR	n_p	n_{np}	*L*	T_p	C_j	D_j	D_L	*P*	Order of primers based on *Dj* and D_L	VI
NKS26	6	1	7	13	0.264	0.736	0.719	3.566	10	6
mSSCIR19	12	0	12	54	0.005	0.995	0.976	42.195	2	48
NKS38	8	0	8	25	0.025	0.975	0.963	26.676	4	12
NKS 23	13	0	13	59	0.000	1.000	0.985	68.907	1	56
NKS34	12	0	12	41	0.052	0.948	0.931	14.479	5	32
mSSCIR16	10	0	10	34	0.094	0.906	0.874	7.937	7	21
SMC703BS	3	2	5	6	0.268	0.732	0.720	3.571	12	0
mSSCIR3	5	0	5	12	0.245	0.755	0.740	3.850	11	6
mSSCIR18	6	0	6	15	0.080	0.920	0.904	10.441	6	0
SMC766BS	4	0	4	9	0.227	0.773	0.760	4.169	9	3
SMC7CUQ	6	3	9	13	0.182	0.818	0.805	5.141	8	5
mSSCIR78	10	0	10	34	0.017	0.983	0.968	31.480	3	22

explain their results. Chromosome numbers were not investigated in our materials. According to Liu *et al.*, (2011) SSR markers developed for one country might not work in another country due to differences in the genetic background of the genotypes. The set of SSR marker used in this research showed a high degree of transferability to the hybrid materials included (100% successfully amplified).

Fig. 1. shows the fingerprint of the 107 SSR loci. The illustration offers a graphic visualization of the variability estimated among the 62 sugarcane genotypes included in the study.

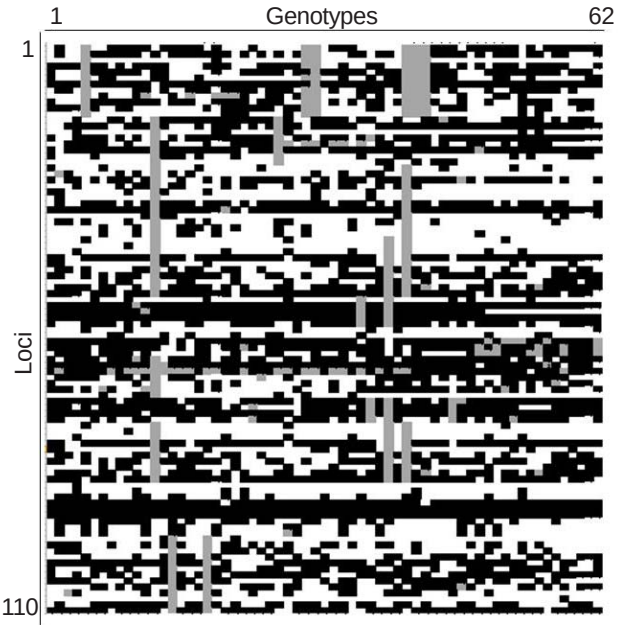

Fig. 1. SSR fingerprints of sugarcane hybrids from INTA Breeding Collection (Argentina). Shared absent bands (0) are shown in black; the white color indicates shared bands, (1) and the grey squares indicate missing data

The analysis of genetic relationships was carried out considering 58 sugarcane genotypes because individuals with more than 10% data missing were discarded. Loci with more than 10% missing data were also removed from the data matrix for the estimation of genetic dissimilarities. A histogram of pairwise dissimilarity for the remaining 58 sugarcane hybrids from the SSR data is presented in Figure 2 and indicates a normal distribution with a mean of 0.46. The dissimilarity coefficients ranged from 0.064 (CP65-357; F97-786) to 0.68 (CP57-614; NA73-1454). The majority of the dissimilarity coefficients were observed between 0.5 and 0.6, and most of the SSR-based pairwise comparisons exhibited genetic dissimilarities higher than 0.53. Table 4 shows the dissimilarity values between pairs of sugarcane hybrids.

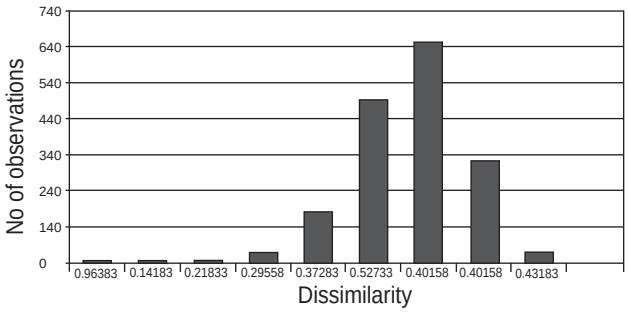

Fig. 2. Frequency of genetic dissimilarity among pairwise combinations of 56 sugarcane accessions based on SSR data.

The bootstrap analysis was summarized with a majority-rule consensus tree, considering the limit of 50% to indicate statistical support for the topology at a particular node (Fig. 3). Eight clusters were supported by more than 50% occurrences. Cluster VII and VIII and several subgroups within clusters I, II and III were supported by the maximum bootstrap score (100%), indicating strong support for these nodes. Cluster I contained the majority of sugarcane hybrids (19 genotypes), with 71% of bootstrap value. Ten of the twelve "TUC" varieties from EEAOC-Tucumán (Argentina) were grouped together, coinciding with their place of origin. The pairs F97-786/CP65-357 and TUC77-42b/F98-70, which were suspected to be duplicate genotypes, showed near expected low dissimilarity (0.064 and 0.13, respectively); and were grouped together, as supported by 100% occurrences. The PIC values were so high that even duplicate genotypes were differentiated at molecular level. In respect to clones TUC77-42 and TUC77-42b the dissimilarity value obtained (0.32) indicate that they are not the same genotype.

The remaining four branches were found to be unresolved (<50%) (LCP85-384, NA78-724, HoCP90-941 and NA63-90).

The number of banding patterns per marker (T_p) was highly variable, from 6 up to 59 (Table 3). For the 12 SSR tested, the total number of banding patterns was 315. Coinciding with the highest number of banding patterns, the microsatellite NKS23 allowed identification of the most unique accessions (56). However, SMC776BS, with only 4 different banding patterns, was able to discriminate 3 genotypes, which was better than mSSCIR18, with 6 banding patterns, which failed to identify any of the 62 sugarcane varieties studied. This information and the discrimination power analysis revealed that the efficiency of a given primer does not depend only on the number of bands or the banding pattern. These

Table 4. Genetic dissimilarities estimated for 58 sugarcane varieties based on SSR markers

	LCP65-384	LCP86-454	LCP85-376	NCo310	NoCP85-845	HoCP92-643	HoCP92-645	HoCP92-624	HoCP89-888	HoCP91-552	HoCP92-631	HoCP91-555	HoCP90-941	US74-1011	US74-1015	US72-1289	L75-33	TCP81-3067	TCP87-368	NA84-3013
LCP65-384	0																			
LCP56-454	0.45	0																		
LCP85-375	0.53	0.35	0																	
NCo310	0.54	0.54	0.33	0																
HoCP85-845	0.52	0.37	0.38	0.51	0															
HoCP92-648	0.38	0.58	0.54	0.5	0.53	0														
HoCP92-645	0.49	0.49	0.54	0.57	0.39	0.45	0													
HoCP92-624	0.4	0.45	0.51	0.52	0.49	0.4	0.44	0												
HoCP89-888	0.5	0.57	0.53	0.54	0.53	0.46	0.45	0.45	0											
HoCP91-552	0.43	0.46	0.46	0.54	0.39	0.35	0.38	0.4	0.38	0										
HoCP92-631	0.51	0.47	0.52	0.52	0.46	0.49	0.37	0.46	0.44	0.53	0									
HoCP91-555	0.53	0.42	0.43	0.51	0.43	0.46	0.45	0.45	0.52	0.47	0.42	0								
HoCP90-941	0.41	0.52	0.6	0.59	0.57	0.49	0.54	0.46	0.58	0.48	0.52	0.55	0							
US74-1011	0.56	0.43	0.5	0.54	0.38	0.52	0.41	0.46	0.44	0.49	0.36	0.41	0.52	0						
US74-1015	0.51	0.47	0.58	0.55	0.49	0.52	0.46	0.46	0.46	0.48	0.42	0.49	0.56	0.37	0					
US72-1282	0.59	0.48	0.51	0.58	0.45	0.53	0.49	0.48	0.5	0.45	0.38	0.54	0.58	0.37	0.44	0				
L75-33	0.41	0.47	0.49	0.55	0.4	0.47	0.4	0.38	0.48	0.48	0.32	0.47	0.49	0.42	0.47	0.44	0			
TCP81-3067	0.43	0.38	0.42	0.49	0.47	0.46	0.48	0.46	0.46	0.38	0.52	0.56	0.43	0.52	0.48	0.54	0.45	0		
TFP87-388	0.48	0.53	0.68	0.63	0.54	0.48	0.45	0.47	0.57	0.52	0.41	0.56	0.43	0.47	0.43	0.52	0.46	0.51	0	
NA84-3013	0.52	0.48	0.43	0.33	0.51	0.48	0.55	0.48	0.57	0.54	0.43	0.47	0.62	0.47	0.51	0.52	0.53	0.51	0.59	0
NA78-724	0.5	0.57	0.53	0.56	0.47	0.53	0.51	0.52	0.43	0.48	0.48	0.54	0.53	0.53	0.55	0.5	0.38	0.53	0.59	0.52
NA84-3471	0.56	0.57	0.55	0.46	0.55	0.43	0.48	0.46	0.54	0.54	0.43	0.55	0.63	0.47	0.55	0.56	0.52	0.49	0.61	0.38
NA63-90	0.43	0.60	0.49	0.41	0.55	0.35	0.55	0.42	0.45	0.47	0.47	0.42	0.47	0.54	0.52	0.59	0.42	0.45	0.54	0.49
NA76-128	0.4	0.55	0.51	0.49	0.52	0.23	0.49	0.4	0.47	0.45	0.47	0.47	0.56	0.46	0.56	0.5	0.47	0.47	0.51	0.4
NA73-2596	0.42	0.52	0.52	0.48	0.47	0.34	0.37	0.39	0.42	0.42	0.39	0.43	0.43	0.49+	0.52	0.51	0.48	0.44	0.49	0.46
NA88-948	0.5	0.46	0.56	0.57	0.55	0.43	0.36	0.37	0.5	0.48	0.38	0.54	0.49	0.51	0.48	0.47	0.47	0.39	0.42	0.5
NA73-1454	0.64	0.57	0.45	0.33	0.54	0.51	0.59	0.55	0.57	0.54	0.6	0.52	0.6	0.56	0.51	0.57	0.58	0.48	0.63	0.44
CP48-103	0.48	0.54	0.61	0.6	0.47	0.37	0.49	0.51	0.56	0.51	0.52	0.48	0.59	0.59	0.49	0.6	0.52	0.54	0.42	0.55
CP68-350	0.37	0.52	0.6	0.59	0.57	0.4	0.35	0.4	0.45	0.43	0.35	0.51	0.49	0.51	0.45	0.52	0.44	0.43	0.33	0.55
CP70-1133	0.44	0.39	0.49	0.48	0.53	0.44	0.47	0.31	0.52	0.44	0.44	0.47	0.47	0.49	0.5	0.53	0.44	0.34	0.53	0.46
CP79-1380	0.4	0.51	0.48	0.44	0.45	0.43	0.47	0.39	0.57	0.48	0.48	0.49	0.46	0.46	0.46	0.51	0.51	0.42	0.55	0.42
CP79-318	0.43	0.54	0.49	0.52	0.47	0.43	0.41	0.34	0.46	0.42	0.39	0.44	0.51	0.5	0.47	0.46	0.3	0.49	0.51	0.51
CP65-350	0.4	0.38	0.46	0.48	0.36	0.44	0.37	0.37	0.45	0.42	0.36	0.34	0.42	0.36	0.46	0.45	0.36	0.4	0.45	0.45
CP57-603	0.49	0.5	0.58	0.53	0.54	0.52	0.46	0.44	0.58	0.44	0.48	0.51	0.5	0.53	0.45	0.54	0.52	0.44	0.49	0.49
CP57-614	0.41	0.51	0.63	0.65	0.59	0.48	0.49	0.4	0.53	0.53	0.48	0.58	0.44	0.5	0.56	0.58	0.45	0.42	0.46	0.61
CP72-2086	0.34	0.41	0.51	0.54	0.48	0.5	0.48	0.38	0.47	0.45	0.47	0.53	0.48	0.52	0.58	0.52	0.39	0.37	0.56	0.52
CP66-346	0.46	0.41	0.37	047	0.34	0.48	0.42	0.5	0.48	0.41	0.44	0.45	0.59	0.44	0.44	0.42	0.44	0.44	0.51	0.49
FAM81-820	0.49	0.58	0.52	0.53	0.49	0.45	0.48	0.42	0.54	0.49	0.44	0.43	0.52	0.49	0.5	0.47	0.38	0.56	0.52	0.54
TUC80-7	0.44	0.4	0.28	0.35	0.33	0.45	0.39	0.44	0.48	0.39	0.41	0.35	0.52	0.35	0.44	0.5	0.35	0.38	0.56	0.38
TUC72-16	0.35	0.52	0.48	0.47	0.42	0.32	0.37	0.33	0.37	0.29	0.42	0.46	0.45	0.47	0.4	0.43	0.29	0.4	0.44	0.5
TUC74-6	0.44	0.5	0.47	0.6	0.49	0.45	0.54	0.41	0.52	0.47	0.43	0.45	0.5	0.55	0.48	0.52	0.42	0.5	0.47	0.44
TUC71-7	0.41	0.45	0.44	0.52	0.32	0.41	0.42	0.45	0.5	0.41	0.44	0.49	0.51	0.45	0.45	0.48	0.33	0.39	0.48	0.5
TUC68-18	0.49	0.5	0.58	0.53	0.57	0.45	0.58	0.52	0.51	0.47	0.5	0.57	0.48	0.57	0.48	0.53	0.45	0.41	0.46	0.55
TUC67-24	0.37	0.42	0.51	0.49	0.33	0.33	0.39	0.39	0.4	0.37	0.35	0.38	0.48	0.38	0.38	0.42	0.41	0.45	0.33	0.45
TUC77-42	0.42	0.45	0.42	0.43	0.39	0.36	0.41	0.33	0.44	0.34	0.43	0.42	0.48	0.44	0.45	0.52	0.32	0.37	0.49	0.52
TUC77-42	0.47	0.43	0.53	0.58	0.25	0.46	0.43	0.46	0.56	0.49	0.43	0.5	0.5	0.43	0.46	0.46	0.39	0.5	0.4	0.52
TUC78-39	0.51	0.59	0.52	0.47	0.55	0.52	0.47	0.38	0.52	0.48	0.52	0.48	0.56	0.52	0.52	0.54	0.51	0.55	0.53	0.46
TUC72-4	0.35	0.49	0.57	0.57	0.4	0.43	0.44	0.4	0.46	0.46	0.46	0.44	0.48	0.51	0.5	0.56	0.51	0.55	0.41	0.58
TUC69-2	0.44	0.45	0.5	0.52	0.3	0.43	0.45	0.39	0.5	0.43	0.37	0.47	0.48	0.44	0.45	0.43	0.32	0.43	0.43	0.52
L91-ZB1	0.35	0.42	0.52	0.53	0.48	0.44	0.43	0.36	0.47	0.44	0.4	0.5	0.45	0.49	0.52	0.52	0.25	0.4	0.45	0.55
RA89-686	0.49	0.57	0.55	0.41	0.55	0.42	0.53	0.52	0.54	0.49	0.5	0.51	0.57	0.55	0.5	0.6	0.55	0.46	0.53	0.38
RA87-2	0.39	0.54	0.56	0.55	0.51	0.31	0.49	0.38	0.55	0.48	0.45	0.5	0.48	0.53	0.48	0.54	0.39	0.39	0.47	0.52
RA91-209	0.51	0.56	0.54	0.5	0.57	0.38	0.55	0.48	0.55	0.5	0.56	0.55	0.5	0.57	0.55	0.55	0.61	0.47	0.46	0.41
RA93-154	0.5	0.58	0.51	0.52	0.59	0.43	0.49	0.47	0.5	0.48	0.53	0.5	0.47	0.58	0.56	0.56	0.51	0.5	0.6	0.52
CP88-1834	0.52	0.37	0.47	0.55	0.36	0.54	0.45	0.46	0.46	0.44	0.37	0.5	0.52	0.43	0.44	0.38	0.38	0.43	0.42	0.51
F98-70	0.5	0.5	0.55	0.6	0.42	0.44	0.48	0.46	0.47	0.47	0.48	0.46	0.57	0.47	0.46	0.48	0.39	0.51	0.53	0.55
F97-395	0.4	0.51	0.58	0.56	0.5	0.48	0.48	0.42	0.45	0.43	0.41	0.52	0.46	0.5	0.46	0.48	0.29	0.42	0.5	0.59
F97-786	0.44	0.52	0.55	0.56	0.44	0.46	0.48	0.44	0.47	0.47	0.34	0.49	0.52	0.43	0.45	0.49	0.25	0.45	0.47	0.56
CF85-357	0.44	0.54	0.57	0.58	0.46	0.47	0.44	0.44	0.44	0.44	0.38	0.52	0.52	0.46	0.45	0.47	0.25	0.48	0.49	0.58
TUC77-426	0.45	0.58	0.63	0.63	0.44	0.47	0.46	0.44	0.5	0.47	0.52	0.54	0.53	0.54	0.47	0.52	0.43	0.51	0.52	0.6

Contd .....

	NA78-724	NA84-3471	NA63-90	NA76-128	NA73-2596	NA88-948	NA73-164	CP48-103	CP68-360	CP70-1133	CP79-1380	CP79-318	CP65-360	CPS7-603	CPS7-614	CP72-2086	CP66-346	FAAM1-820	TUCRO-7	TUC72-16	TUC74-6	TUC71-7	TUC68-18	TUC67-24
NA78-724	0																							
NA84-3471	053	0																						
NA63-90	0.46	0.37	0																					
NA76-128	0.48	0.33	0.35	0																				
NA73-2596	0.41	0.38	0.34	0.34	0																			
NA33-948	0.54	0.46	0.56	0.47	0.55	0																		
NA93-1454	0.59	0.52	0.45	0.55	0.56	0.55	0																	
CP48-103	0.62	0.55	0.47	0.46	0.45	0.54	0.6	0																
CP68-350	0.51	0.51	0.47	0.43	0.34	0.25	0.65	0.47	0															
CP70-1133	0.53	0.46	0.45	0.44	0.44	0.43	0.54	0.48	0.48	0														
CP79-1380	0.59	0.44	0.36	0.46	0.39	0.49	0.55	0.51	0.46	0.35	0													
CP79-318	0.42	0.46	0.3	0.48	0.44	0.53	0.49	0.49	0.48	0.43	0.39	0												
CP65-350	0.53	0.52	0.41	0.42	0.41	0.48	0.54	0.48	0.44	0.31	0.33	0.43	0											
CP57-603	0.64	0.53	0.49	0.55	0.51	0.42	0.52	0.57	0.43	0.44	0.44	0.5	0.35	0										
CP57-614	0.55	0.56	0.55	0.55	0.36	0.53	0.68	0.56	0.33	0.47	0.45	0.53	0.48	0.56	0									
CP72-2056	0.47	0.52	0.5	0.47	0.4	0.48	0.61	0.52	0.49	0.25	0.51	0.44	0.57	0.55	0.5	0								
CP66-346	0.47	0.52	0.49	0.5	0.47	0.46	0.45	0.46	0.52	0.5	0.5	0.43	0.4	0.51	0.53	0.52	0							
FAM81-820	0.49	0.51	0.45	0.47	0.44	0.52	0.5	0.53	0.51	0.47	0.47	0.37	0.4	0.57	0.51	0.48	0.41	0						
TUC80-7	0.48	0.4	0.37	0.44	0.42	0.49	0.48	0.53	0.52	0.48	0.57	0.39	0.51	0.44	0.54	0.51	0.29	0.42	0					
TUC72-16	0.4	0.45	0.33	0.41	0.37	0.48	0.46	0.42	0.41	0.47	0.4	0.21	0.36	0.51	0.43	0.44	0.32	0.37	0.33	0				
TUC74-6	0.58	0.58	0.44	0.42	0.45	0.48	0.59	0.44	0.49	0.5	0.47	0.42	0.41	0.51	0.54	0.52	0.47	0.45	0.44	0.44	0			
TUC71-7	0.55	0.49	0.41	0.36	0.44	0.52	0.57	0.44	0.48	0.51	0.42	0.42	0.34	0.46	0.56	0.49	0.34	0.45	0.23	0.28	0.33	0		
TUC68-18	0.58	0.53	0.35	0.48	0.44	0.54	0.52	0.44	0.47	0.57	0.52	0.51	0.46	0.53	0.54	0.54	0.48	0.48	0.45	0.39	0.37	0.52	0	
TUC67-24	0.46	0.48	0.44	0.38	0.32	0.46	0.54	0.24	0.39	0.42	0.45	0.43	0.36	0.53	0.46	0.4	0.38	0.48	0.4	0.22	0.41	0.36	0.41	0
TUC79-9	0.47	0.51	0.4	0.47	0.38	0.45	0.49	0.53	0.35	0.41	0.41	0.32	0.35	0.46	0.44	0.43	0.42	0.44	0.3	0.23	0.51	0.37	0.46	0.38
TUC77-42	0.54	0.59	0.54	0.46	0.51	0.5	0.63	0.45	0.49	0.5	0.43	0.5	0.34	0.56	0.58	0.53	0.42	0.53	0.39	0.41	0.46	0.35	0.53	0.34
TUC78-39	0.5	0.5	0.44	0.52	0.44	0.5	0.49	0.61	0.45	0.45	0.43	0.35	0.49	0.45	0.51	0.57	0.5	0.47	0.45	0.44	0.57	0.56	0.59	0.56
TUC72-4	0.53	0.6	0.44	0.51	0.45	0.51	0.63	0.58	0.44	0.53	0.49	0.44	0.57	0.56	0.48	0.52	0.44	0.57	0.43	0.35	0.45	0.42	0.48	0.28
TUo69-2	0.52	0.54	0.48	0.45	0.42	0.45	0.57	0.45	0.42	0.43	0.43	0.44	0.52	0.54	0.41	0.5	0.35	0.42	0.34	0.27	0.41	0.25	0.44	0.33
L91-281	0.52	0.53	0.48	0.48	0.43	0.41	0.61	0.54	0.38	0.36	0.46	0.39	0.35	0.46	0.28	0.37	0.45	0.43	0.46	0.55	0.47	0.39	0.52	0.4
RA59-686	0.51	0.43	0.43	0.44	0.33	0.44	0.47	0.53	0.43	0.47	0.49	0.52	0.52	0.51	0.45	0.56	0.49	0.55	0.69	0.42	0.53	0.52	0.49	0.45
RA87-2	0.57	0.46	0.4	0.59	0.4	0.46	0.54	0.47	0.48	0.39	0.39	0.39	0.4	0.48	0.41	0.42	0.46	0.35	0.46	0.36	0.35	0.34	0.41	0.43
RA91-209	0.57	0.47	0.44	0.4	0.32	0.42	0.54	0.52	0.47	0.54	0.4	0.52	0.54	0.56	0.5	0.58	0.51	0.52	0.51	0.49	0.44	0.44	0.48	0.45
RA93-154	0.55	0.54	0.46	0.44	0.47	0.43	0.5	0.59	0.53	0.51	0.53	0.38	0.51	0.56	0.54	0.57	0.48	0.47	0.48	0.44	0.49	0.46	0.58	0.57
CP85-1534	0.5	0.55	0.52	0.48	0.45	0.42	0.56	0.52	0.43	0.48	0.48	0.45	0.41	0.52	0.46	0.49	0.36	0.4	0.42	0.36	0.4	0.35	0.44	0.39
P98-70	0.54	0.61	0.54	0.45	0.53	0.52	0.58	0.54	0.5	0.51	0.53	0.45	0.41	0.54	0.55	0.55	0.54	0.43	0.39	0.34	0.46	0.35	0.55	0.44
P97-395	0.4	0.54	0.45	0.56	0.47	0.45	0.58	0.58	0.44	0.36	0.45	0.25	0.4	0.49	0.38	0.38	0.43	0.42	0.47	0.3	0.52	0.48	0.5	0.46
P97-786	0.59	0.51	0.39	0.43	0.41	0.5	0.51	0.5	0.46	0.44	0.49	0.35	0.59	0.50	0.49	0.43	0.45	0.38	0.41	0.35	0.46	0.35	0.43	0.41
CP65-357	0.36	0.55	0.45	0.47	0.44	0.53	0.59	0.59	0.48	0.46	0.52	0.5	0.39	0.55	0.52	0.45	0.42	0.33	0.42	0.31	0.46	0.55	0.43	0.44
TUC77-42b	0.6	0.62	0.55	0.53	0.52	0.53	0.61	0.49	0.5	0.51	0.51	0.46	0.44	0.55	0.54	0.51	0.5	0.44	0.47	0.37	0.47	0.38	0.5	0.44

	TUC79-9	TUC77-42	TUC78-39	TUC72-4	TUC69-2	L91-281	RA89-686	RA87-2	RA91-209	RA93-154	CP88-1834	F98-70	F97-395	F97-786	CP85-357	TUC77-42b
TUC79-9	0															
TUC77-42	0.4	0														
TUC78-39	0.41	0.58	0													
TUC72-4	042	0.33	0.55	0												
TUC69-2	0.36	0.35	0.52	0.33	0											
L91-281	0.32	0.47	0.46	0.41	0.31	0										
RA89-686	0.43	0.6	0.43	0.53	0.46	0.49	0									
RA87-2	0.37	0.47	0.48	0.46	0.32	0.27	0.45	0								
RA91-209	0.53	0.56	0.5	0.54	0.46	0.49	0.38	0.37	0							
RA93-154	0.44	0.58	0.41	0.59	0.47	0.42	0.43	0.38	0.42	0						
CP55-1834	0.42	0.39	0.45	0.47	0.26	0.32	0.52	0.35	0.41	0.47	0					
F96-70	0.37	0.28	0.51	0.4	0.35	0.39	0.53	0.41	0.55	0.42	0.33	0				
F97-395	0.39	0.52	0.39	0.45	0.37	0.23	0.49	0.35	0.56	0.42	0.37	0.44	0			
F97-786	0.38	0.45	0.49	0.47	0.3	0.35	0.48	0.37	0.53	0.46	0.36	0.43	0.22	0		
CP85-357	0.41	0.48	0.47	0.47	0.31	0.38	0.51	0.37	0.55	0.44	0.33	0.4	0.2	0.06	0	
TUC77-42b	0.41	0.32	0.51	0.37	0.38	0.42	0.56	0.39	0.57	0.49	0.4	0.13	0.44	0.45	0.41	0

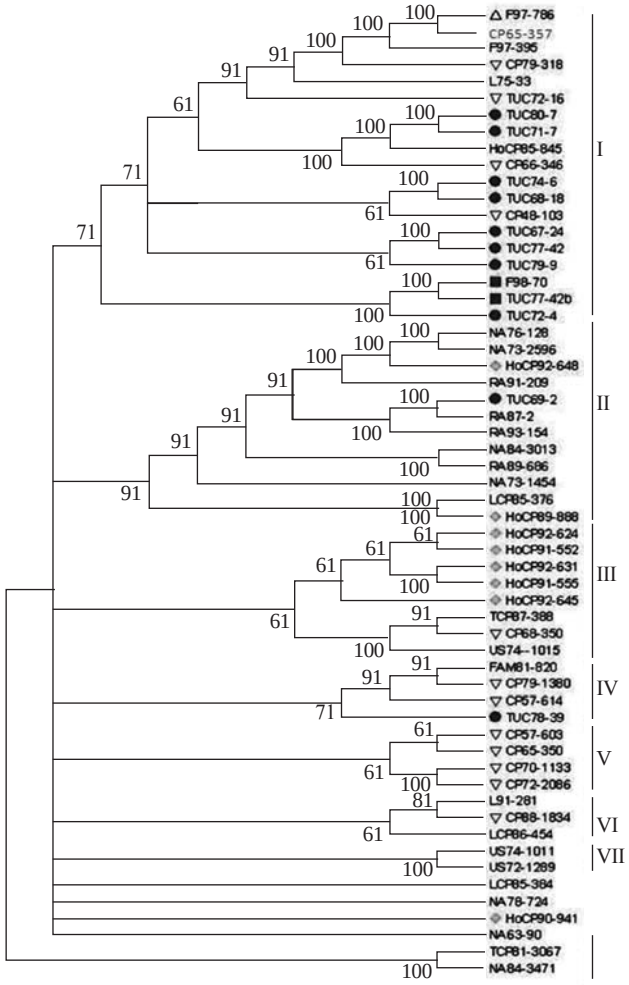

Fig. 3. Unrooted phylogenetic tree depicting the genetic relationships among the *Saccharum* based on genetic dissimilarity coefficients. The numbers indicate the percentages with which a given branch is supported in 1000 bootstrap replications. The consensus tree only shows a topology but does not display branch lengths. ▲ Indicate two duplicate accessions in the collection. ● ▽ ◊ Indicates accessions from EEAOC-Tucumán (Argentina), Canal Point (Florida-Houma)-USDA and Houma (Louisiana) Canal Point (Fla)-USDA, respectively.

Table 5. Primers optimal combination for the identification of a set sugarcane accessions from INTA Breeding Collection. Joint confusion probability (C_k) for the five most discriminating primers. Theoretical number of non-differentiated pairs of genotypes (X_k) for a given combination of k primers on a set of 62 accessions (1891 pairs) estimated under the hypothesis of independence of the considered primers patterns. Because cero values for Cj were found, a correction was applied (C_{Jc}=C_jx0.001)

SSR	C_j	C_{jc}	C_k	X_k
NKS 23	0.000	0.001	1.00×10^{-3}	4,3
mSSCIR19	0.005	0.006	6.00×10^{-6}	2.5×10^{-2}
mSSCIR78	0.017	0.018	1.08×10^{-7}	4.6×10^{-4}
NKS38	0.025	0.026	2.00×10^{-9}	8.5×10^{-6}
NKS34	0.052	0.053	1.48×10^{-10}	6.3×10^{-7}

findings are consistent with those of Tessier *et al.*, (1999). Using random amplified polymorphic DNA, they found that discriminating power depends on the frequency differences between patterns generated by the primers. They found that a primer has maximal discriminating power when it generates patterns at the same frequency (the isofrequency situation). In our case, the primers with the maximal discriminating power were NKS23 and mSSCIR19, which generated different numbers of banding patterns but were nearly isofrequent (0.0003 to 0.01). In contrast, primers NKS16 and SMC703BS showed very different frequencies (0.06 to 0.44) in their banding patterns, which account for their lower discriminating powers, confirming that the efficiency of SSR loci is also subject to this rule.

Low values for confusion probability (Cj) were obtained. They ranged from 0 (NKS23) to 0.268 (SMC703S). As it is negatively correlated, the microsatellite NKS23 showed the highest value of discriminating capacity (Dj) supporting the significant utility of this SSR primer in sugarcane variety identification. However, there are other primers with high Dj values (>0.9) that are also effective (Table 3).

The DL value ranked from 0.719 to 0.985, with a mean of 0.865. DL value is an extension of the Polymorphism Information Content (PIC) (Anderson *et al.*, 1993), which is determined by the frequencies of different banding patterns generated by a primer. Our results are in the range of those values reported by Pan (2006) and Creste *et al.*, (2010), 0.75 and 0.82 respectively. Moreover, the PIC values for SSRs used in this study were higher than those mean PIC values reported by Marconi *et al.*, (2011) and Banumathi *et al.*, (2010), 0.69 and 0.66 respectively. Liu *et al.*, (2011) recognized that the PIC value of any SSR marker is not constant and change with the number of samples, the more diverse the panel of genotypes, the higher the PIC values. Banumathi *et al.*, (2010) and Marconi *et al.*, (2011) worked with a panel of 48 and 18 clones respectively. The ability of a few markers to generate unique genetic profiles in sugarcane accessions is due to the complex polyploid nature of the genome, which allowed the detection of several allelic types in a single accession. DL values close to 1 demonstrated that, in spite of the low number of primers (12) used, these SSRs were sufficiently polymorphic and informative and should be useful in registration of sugarcane varieties and in genetic identity tests.

High values for effective number of patterns (*P*) per unit assay were detected. The highest value (68.90) corresponds to NKS23. Belaj *et al.,* (2003) showed that this parameter indicates the size of an ideal population in which all of the individuals can be distinguished. In our case, with NKS23, almost 69 parameters can be obtained when the population size tends to infinity (up to 69 varieties can be identified with the same primer). Relatively high *P* values illustrate SSR primer discrimination capacity when handling a large number of samples in sugarcane, which is very important for germplasm collections management where numerous genotypes need to be accurately characterized and identified.

The primers NKS 23, mSSCIR19, mSSCIR78, NKS38 and NKS34 were selected based on their high discriminating powers. The joint confusion probabilities (C_x) and theoretical numbers of indistinguishable pairs (X_k) estimated with these primers showed that the combination of the first two primers (NKS23 and mSSCIR19) is effective for discriminating the 62 sugarcane genotypes with a cumulative confusion probability value of 6.00 x 10^{-6}, with just 0.025 pairs of accessions from the theoretical 1891 pairs indistinguishable (Table 5). Increasing the number of combinations of primers decreases the theoretical number of indistinguishable pairs, but the cost of the analysis increases. For this reason, the most informative primers should be chosen to reduce the cost and the time of analysis and ensure reliable varietal identification.

Conclusions

SSRs were useful for assessing genetic variation and genetic relationships of materials in a sugarcane breeding collection at INTA. The polyploid nature of the sugarcane genome allows the detection of numerous SSR banding patterns, and thus a small number of microsatellite markers generate unique genetic profiles in sugarcane genotypes. The high PIC values and the high discriminating capacity of SSR primer combinations tested in this study suggest that SSRs would be suitable for use in genotype identification.

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Population Structure Analysis of Cashew (*Anacardium occidentale* L.) in Kerala -the Region of Introduction in India

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Cashew (*Anacardium occidentale*) entered the Malabar Coast of Kerala in 16th century along with the Portuguese. More than five centuries of existence in this area has acclimatized this crop to this region. The present study reveals the population genetic structure of cashew in Kerala through AFLP marker analysis. The germplasm accessions specific to the locality were used for the study. AFLP analysis gave moderately high Gst (0.23) and Fst (0.31) values which showed moderate to high genetic diversity. Genetic load developed by the cross pollination and use of non descript seeds have widened the genetic variation in addition to the few introductions. Population structure analysis confirmed the uniqueness of the Kannoore population of cashew in the northern districts of Kerala near the port of entry of the initial introductions. This study shows that moderate to high genetic diversity observed in cashew populations of Kerala has raised this region of introduction to the status of secondary centre of origin of cashew.

Key Words: AFLP, Cashew, Genetic diversity, Population structure

Introduction

Kerala had established itself as a major spice trade center since 3000 BC and had direct contact across the Arabian Sea with all the major sea ports in Red Sea and the Mediterranean, extending to the Far East (Menon, 1967). 'Muziris' the ancient port city of Malabar was said to be the greatest trading centre of the east in the ancient world. It had productions of pepper, cinnamon, cardamom, ginger and other spices. Through this port, south India had trade links with the West Asia, and Europe (Craig, 1997). Vasco da Gama's voyage to Kerala from Portugal in 1498 was largely motivated by Portuguese determination to break the Arabs' control over trade of spices grown in Kerala. In return the navigators provided new crops like cashew, rubber cocoa etc.to the west coast (Ravenstein, 1898).

Cashew is one of the few fruit crops normally grown from seeds. This is a native of eastern Brazil and introduced to the east along with other commercial crops like rubber, coffee, tea etc. by the Portuguese nearly five centuries back. The first introduction of cashew in India was made in Malabar coast from where it spread to other parts of the country. The vernacular name "kasu mavu" originated from the Portuguese name'caju'. Since the Portuguese entered kerala through the ports in Kochi and Kozhikode these areas are presumed to be the portals of cashew entry in Kerala. The trees were well adapted to the region, and became acclimatized. The commercial exploitation began from the early 1960's and due to the absence of high yielding varieties and suitable propagation techniques, non descript seeds and seedlings were used for planting purposes. Because of its adaptive ability in wide range of agro climatic conditions cashew has become a crop of high economy and attained the status of an export oriented commodity bringing considerable foreign exchange to the country. In addition to the few early introductions, import as seeds by the foreign traders - handed over to planters also contributed the genetic diversity of the cashew plantations in Kerala.

Success of the crop improvement programmes depend on the variability available and the genetic diversity existing in the populations. Germplasm maintained in a particular area includes all the variable accessions with unique characters prevailing in that locality (Frankel, 1984). Characterization and cataloguing of these types is a prerequisite to select the superior ones for further crop improvement programmes. Analysis of genetic diversity in germplasm collection can facilitate reliable classification and identification of core groups with possibility to use for specific breeding programmes (Brown, 1989).

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In Kerala more than 50% of the cashew cultivation is concentrated in northern and central districts (Fig.1). In this state cashew germplasm is maintained at three locations under Kerala Agricultural University. The regional Agricultural Research Station at Pilicode in Kannoore district maintains the collection from northern districts of Kerala. The research station at Anakkayam in Malappuram district collects and maintains novel genotypes from the central districts. Cashew research station at Madakathara maintains germplsm collections from central and southern districts mostly from Kottarakara and Kollam. Germplasm accessions from these three field genebanks were undertaken for the study.

Molecular markers had been proven as a powerful tool for analyzing genetic diversity in cashew (Mneney *et al.*, 2001; Dhanraj *et al.*, 2002; Archak *et al.*, 2003a, 2003b and 2009; Thimmappiah *et al.*, 2009; Jayalekshmy *et al.*, 2010). However the previous works were based on RAPD markers and have reported low genetic diversity (Mneney *et al.*, 2001; Dhanraj *et al.*, 2002). Archak *et al.*, (2003b) have compared RAPD, ISSR and AFLP markers for their efficiency in discriminating cashew genotypes and found AFLP markers to be the most efficient.

The major objective of molecular ecological research is to study the genetic structure of populations (Bensch and Akeson, 2005). Evaluating population structure is of considerable interest because it is a precursor to answering many other questions such as estimating migration, identifying conservation units and specifying phytogeographic patterns (Manel *et al.*, 2005). So far AFLPs have been used in a range of applications to assess population structure from small to large scales (Hardy *et al.*, 2006; River-Ocasio *et al.*, 2006; Archak *et al.*, 2009). Bayesian analysis of population structure using the model based approach of Pritchard *et al.*, (2000) provides a stratification pattern consistent with the observations derived from the traditional Fst approach.

Fig. 1. Location site of cashew populations under study

Archak *et al.* (2009) have utilized these markers to asses the population structure of Indian cashew. Kerala is the state with earliest histories of cashew introduction and popularization. Even now it has the largest area under cashew cultivation. In this study cashew germplasm of Kerala is being assessed based on AFLP markers to study the extent of variability and to know the population structure of cashew in Kerala which will throw light to the early introduction and spread of cashew in Kerala

Materials and Methods

Sixty cashew accessions maintained in the three research stations were selected for the study. Depending on the number of original collection 30 accessions were selected from Madakkathara and 15 each from Anakkayam and Pilicode (Table 1). The accessions from one location were considered as one population. These accessions were genetically analysed with AFLP markers generated from 5 primer pairs.

Plant DNA Isolation

A novel and efficient protocol for isolation of good quality DNA using CTAB (Cetyl trimethyl ammonium bromide) was standardized with extraction buffer (3% CTAB, 100mM Tris HCl (pH-8.0), 20mM EDTA,1.4M NaCl, 3%[w/v] Polyvinyl polypyrrolidone (PVP) and 1% [v/v] β-mercaptoethanol The DNA was pelletted in ethanol and dried pellet dissolved in100µl TE buffer. Good quality DNA, which could be used for AFLP was obtained by this cost effective method.

AFLP Analysis

Genomic DNA (60ng) was digested with *EcoRI & MseI* and double stranded adaptors legated to the fragments ends. This was followed by a pre-amplification step using non-selective primers. Selective amplifications were performed on the pre-amplified fragments mixture using a total of six primer combinations. Primers and other reagents provided in the AFLP Kit (Invitrogen) were used following their protocol. Only the *EcoRI primer* was radiolabeled with γ [32P] ATP. Amplified products were separated by denaturing 4% polyacrylamide gel electrophoresis (PAGE) visualized by autoradiography

Table 1. List of cashew accessions
(Accessions from Cashew Research Station Madakathara (1-30), Accessions from cashew research stations Anakkayam (31-45) and Pilicode (46-60))

Sl. No.	Name of accessions	Sl.No.	Name of accessions
1	Rajamundry	31	ALGD1-1
2	K-3-2	32	ABD-2-2
3	KTR-1-254	33	T-8-A
4	Anakkara	34	BRZ-18-1
5	Kottarakkara	35	NLR-1-1
6	P-10-1	36	K-12-1
7	Ullikkal-6	37	PTR-2-1
8	A/26/2	38	UL-3-2
9	Kelakam-1	39	CRPT-1-1
10	B-2-48	40	NL-2-1
11	Payam-1	41	K-27-2
12	Kankady	42	K-28-2
13	Peravoor-2	43	T-30-4
14	K-30-1	44	ABD-2-1
15	Pu-2	45	K-6-2
16.	Vettore-56	46	PCC-9
17	PU-8	47	Mdp-2
18	Rajapalayam	48	BLM-3
19	PTR-1	49	TDB-1
20	Payam-2	50	Thumbassery
21	P-3-2	51	PCK-1
22	Vapala	52	ADR-2
23	Ambayathode	53	CGR/1G-2
24	K-2	54	PU-1
25	B-2-44	55	AR-2
26	Ullikkal-1	56	PRL-1
27	K-3-1	57	MK-1
28	K-1	58	MLR-1
29	Anandappilly	59	KD
30	K-10-1	60	PT-1

& phosphor imaging methods. Five AFLP primer pair combinations were used in the study these are

1. E-ACG, M-CAT 2. E-AAG, M-CAA
3. E-ACA, M-CAT 4. E-ACT, M-CTC
5. E-AGG, M-CAC 6. E-ACT, M-CAC

Data Analysis

Each AFLP band was treated as a unit character and scored manually using independent binary codes (1 for presence and 0 for absence) sand 1/0 matrices were generated. Independently scorings were repeated and conflicting data were eliminated from the analysis. The resulting binary data matrix was used to compute a parwise distance matrix using the DICE coefficient utilizing the software WINBOOT (Yap and Nelson, 1996). The distance matrix was subjected to cluster analysis employing UPGMA using SAHN (Sequential Aglomerative Heirarchial and nested cluster) module of the software NTSYS pc (Rohlf, 2002). The robustness of the clusters was estimated by performing a bootstrap analysis on 1000 data replicates using WINBOOT (Yap and Nelson, 1996). Principal coordinate analysis was conducted using the EIGEN procedure of NTSYS pc in order to cluster the genotypes. The value of each of the five primer pairs was assessed using two indices PIC which is same as diversity index (DI) and Rp (Prevost and Wilkinson, 1999). PIC was estimated as PIC= $\sum(1\text{-pi}^2)/n$ where n is the number of band positions analyzed in the set of accessions, pi is the frequency of the i^{th} pattern. The ability of the primers to distinguish between accessions was assessed by calculating their resolving power as, Rp= $\sum$Ib where Ib is the band informativeness and Ib=1-(2x0.5-pi) where pi is the proportion of individuals containing the band (Prevost and Wilkinson, 1999). Marker Index was calculated as the product of DI and EMR (Effective multiplex ratio). EMR of a primer is defined as the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay (Milbourne *et al.*,1997).

POPGENE software version 1.32 (Yeh *et al.,* 1997) was used to estimate the percentage of polymorphic loci, observed number of alleles, shanon information index (I) (Lewontin, 1972), Nei's gene diversity (h) (Nie, 1973) and geneflow (Nm) from the matrix generated from AFLP markers. Arlequin version 3.11 was used to evaluate the degree of genetic differentiation among populations using Gst statistics and Fst statistics.

The software Structure version 2.2 was used for inferring population structure (Pritchard *et al.,* 2003). This programme facilitates testing the veracity of the hypothesis about the number of populations by calculating the probability of the data for each hypothesis. The programme assumes that the markers are not in linkage disequilibrium and that they exhibit co-dominance, however there is a provision to estimate population structure based on the dominant markers as well. This was done by designating AA/Aa individuals as (1,-9), and an aa individual as (2,-9), where-9 was the value for the missing data. The programme" STRUCTURE" was run for many values of'k' for a burn in period of 10,000.

Results

A total of 277 amplicons were produced by the six primer pairs and of these 163 amplicons were polymorphic giving 58.84% polymorphism. The number of products produced by each primer pair combination and the number of polymorphic products is given in Table.2. Primer pair E-ACG+M-CAT gave highest number of products with

highest polymorphism. The total polymorphism ranged from 40 to 82.35 %. This primer pair gave the highest value for resolving power and marker Index (Table 2). So this primer pair can be effectively used for analyzing cashew genotypes with AFLP.

Table 2. AFLP primers, number of amplicons and percentage of polymorphism

Sl. No.	Primer Name	Number of products	Number of polymorphic products	Polymorphism (%)	PIC	Rp	MI
1	E-ACG+M-CAT	68	56	82.35	0.8432	29.94	29.94
2	E-AAG_M-CAA	66	28	42.42	0.8745	15.85	15.85
3	E-ACA+M-CAT	58	26	44.82	0.770	13.18	13.18
4	E-ACT+M-CTC	49	37	75.51	0.862	18.80	18.80
5	E-AGG+M-CAC	36	16	44.40	0.803	8.15	8.15
TOTAL		277	163	58.84			

PIC - Polymorphic information content; RP - Resolving Power; MI - Marker Index

The dendrogram constructed based on the AFLP markers (Fig. 2) indicated that the accessions selected for the study had below 83% similarity. The accessions from germplasm maintained at Pilicode were placed together. These accessions clustered with the accessions from other two stations only at 21% similarity. Five accessions from Anakkayam also clustered along with them with 40% similarity. Accessions from Madakathara and Anakkayam clustered at 30% similarity. Nearly 80% similarity was shown between the accessions IG-2 & M-1 (53 & 57) and KD & PT-1 (59 & 60). These accessions were from Pilicode. The study of genetic divergence with AFLP markers showed no duplicates in the germplasm maintained at three locations. Only below 41% similarity was noted among the different collections. Thumbassery is a dwarf accession with trailing branches and a unique morphotype but this variety showed a close association with TDB-1 another member of the same population. The bootstrap values above 50 given at the indices of the dendrogram. Principal coordinate analysis confirmed the clustering in dendrogram (Fig. 3).

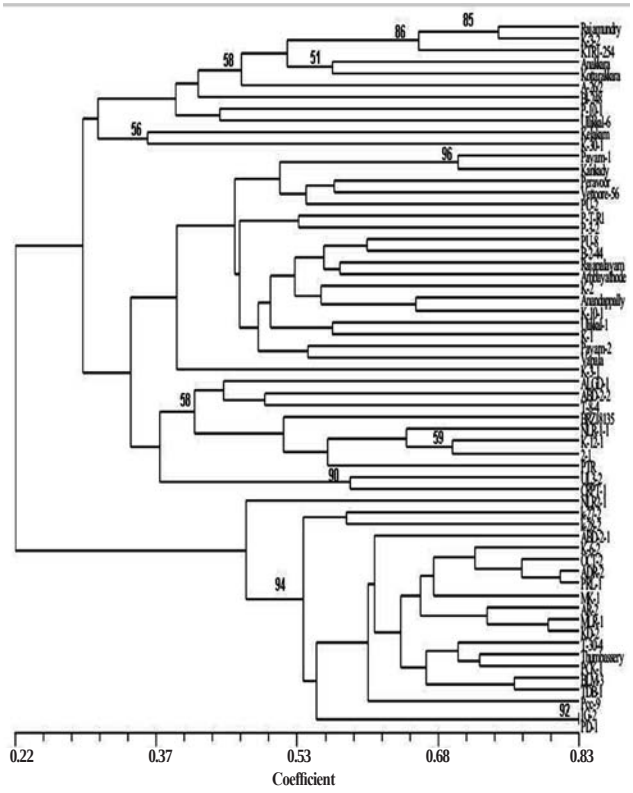

Fig. 2. Dendrogram constructed based on the AFLP scores (Bootstrap values above 50 at nodes)

In this study collections from the three centers were analysed as three populations, since they are the representatives from three distinct geographic zones of Kerala. Germplasm accessions from Madakathara (representing South and Central Kerala) was taken as population-I, accessions from Anakkayam (representing Central Kerala) as Population II and the accessions from Pilicode (representing North Kerala) was taken as Population III. Population analysis using Popgene was done and the effective number of alleles (Kimura and Crow, 1964), gene diversity (Nei, 1973), Shanons information index (Lewontin, 1972), the number of polymorphic loci,G_{st} and gene flow (Nm) across the population calculated using POPGENE and given in Table 3. Neis genetic diversity is highest for population III and Shanon information index was high for population I. Neis genetic identity and distance of the populations derived in the same analysis is given in Table 4. The AMOVA and Fst values calculated using the software ARLEQUIN is given in Table 5 and Table 6, respectively. Significantly high F_{ST} value is obtained in the analysis. Genetic difference among the three populations were significant with average F_{ST} 0.30. Largest pair wise

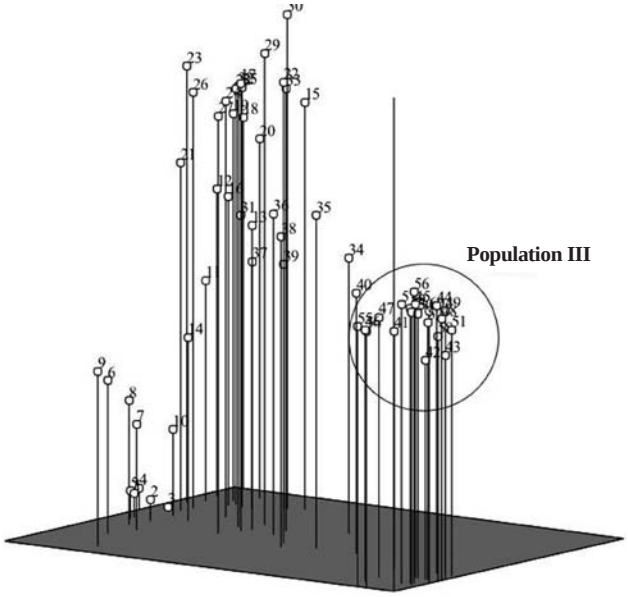

Fig. 3. Ordination projection of cashew genotypes showing the clustering of genotypes in Population III

F_{ST} was noted between population I and III. The lowest level was between I and II. (Table 4). The AMOVA from Population analysis showed that the within population variability was more than the inter population variability (Table 5). In structure analysis independent runs performed for different 'k' values (2, 3, 4) (Fig. 4) and in all the runs, population III was intact with least admixture. Fig.5 shows the triangular distribution at K=3 in structure analysis.

Discussion

The population analysis showed that the Neis genetic diversity across the populations was 0.23. Nies (1973) have reported that this value ranges from 0-0.5 for moderate diversity. Hence the diversity of Kerala cashew can be considered as moderate the same was reported by Archak *et al.*, 2003(b) with respect to Indian cashew.

AFLP based Neis genetic diversity estimates of some of the tree species in their native habitat were also found to be around this value as 0.225 for Belgian wild apple (Coart *et al.*, 2003) 0.243 in Chinese tree hibiscus (Tang *et al.*, 2003), 0.29 in Flemish oak (Coart *et al.*, 2002), 0.342 in Nicaraguan pines (Diaz *et al.*, 2001), 0.193 in Australian mangrove (Maguire *et al.*, 2002).

Table 3. Population analysis of the cashew genotypes

Population	h	I	NPL	PPL	Gst	Nm
I	0.241	0.3728	135	88.24		
II	0.1920	0.3008	110	71.90		
III	0.833	0.1282	43	28.10		
Across population	0.23	0.368	151	98.69	0.20	1.99

I- Accessions in Madakathara II- Accessions in Anakkayam III- accessions in Pilicode

h- Neis genetic diversity I- Shannons information Index

NPL- Number of polymorphic loci PPL- Percentage of Polymorphic loci

Table 4. Nei's Original Measures of Genetic Identity and Genetic distance

pop ID	1	2	3
1	****	0.9482	0.8753
2	0.0532	****	0.9488
3	0.1332	0.0525	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table 5. AMOVA of the populations

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	2	331.683	7.89637 Va	30.75
Within populations	57	1013.733	17.78480 Vb	69.25
Total	59	1345.417	25.68116	

Fixation Index FST : 0.30748

Fst measures the heterozygote defecit relative to its expectation under Hardy – Weinberg equilibrium (Hartl and Clark, 1997). For the interpretation of Fst it has been suggested that a value between 0.05 and 0.15 moderate differentiation 0.15-0.25 greater differentiation and values above 0.25 very great genetic variation (Wright, 1978; Hartl and Clark, 1997). In this study the fixation index was 0.3078 (Table 5). So the populations can be expected to have vast variation. Gene flow in this analysis is substantially high 1.9876 denoting a migration between sub- populations.

The dendrogram constructed based on the AFLP markers showed that none of the cashew accessions had a similarity beyond 80% which denotes that the collection doesn't have any duplicates. The cashew genotypes from the population III clustered together at 60% similarity this showed the uniqueness of the cashew in this part of Kerala (Kasargod and Kannoore districts) the clustering of the accessions from this region had a bootstrap value above 94 (Fig. 1). The other two populations did not show a separate clustering. Ordinate projection of the genotypes also shows the clustering of the genotypes of population III (Fig. 2).

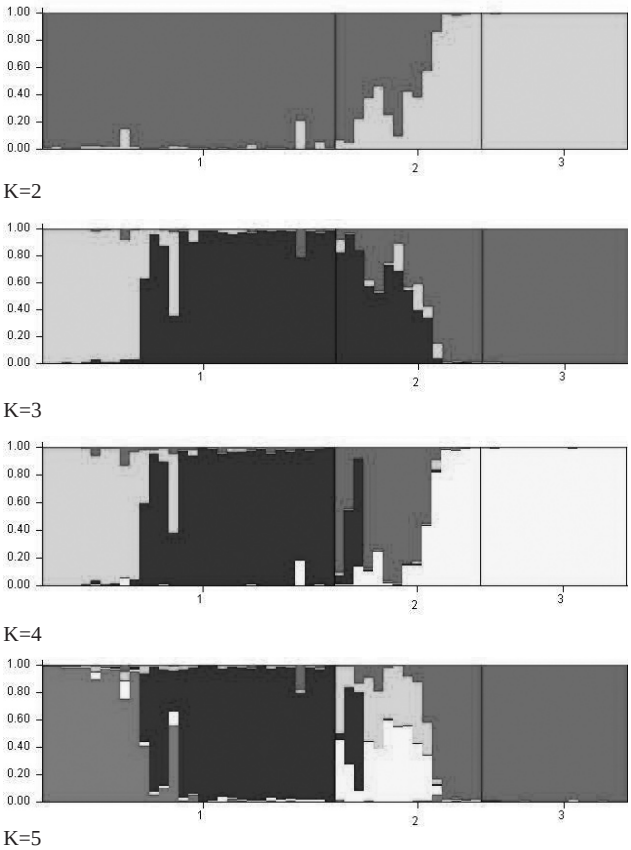

Fig. 4. Estimated population structure of Kerala cashew at different values of 'k'

Intactness of the population III in all the 'k' values shows the uniqueness of the cashew of that region. Archak *et al.*, (2009) reported no population differentiation while studying Indian cashew in different states and they also found shared alleles by all the populations. In this study shared alleles were seen only between two populations. Gst value was also high and the AMOVA showed considerable variation among the populations. The third population which did not have any shared alleles had accessions mostly from Kannoore and Kasargod districts which were the regions of first introduction. The other two populations include collection from the central districts (Pop II) and from southern districts (Pop II). These collections would have included introduced material. Cashew came to Kerala as an introduced crop but over the decades of cultivation it has well acclimatized to the Kerala climate and soil. In addition to the few initial introductions at the Malabar coast by the portugese there would have been more entries through the other ports on the west coast of Kerala and the import by the industrialists. Cashew being a cross pollinated crop the recombinant seedling progenies would have raised the genetic divergence from low to moderate or even high status.

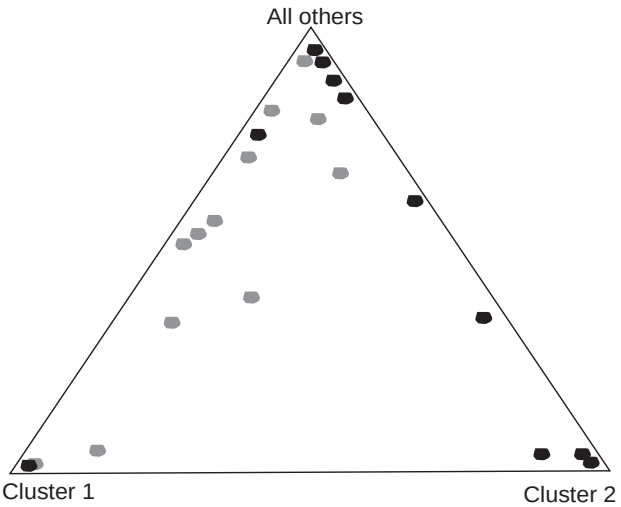

Fig. 5. Triangle plot of the population structure at k=3

Earlier studies have revealed the existence of wide morphological diversity in cashew accessions in India Rao *et al.* (1998), Swamy *et al.* (1998), Jayalekshmy and John, 2004). Archak *et al.* (2009) indicated the existence of substantial genetic diversity in India to raise it to the status of secondary centre of cashew diversity. This study also supports that view and the secondary centre of origin can be the 'Malabar coast' to be more precise. Cashew in kerala especially in Malabar Coast is a typical example of a crop introduction raised to the status of secondary centre of origin near the port of entry through years of acclimatization.

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Storability of Primed Seeds of Brinjal (*Solanum melongena*)

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Studies were conducted to study the effect of priming on storability of brinjal variety Bagyamati. Four different priming methods *viz.*, hydro priming, halo priming, sand matrix priming and osmo priming were used. The storage studies were conducted for 11 months after imposing the priming treatment. The results revealed that viability of primed seeds were dependent on the method of priming. However, irrespective of method all the priming methods were superior to control seeds throughout the storage period. Among the protocols studied sand matrix priming (80% water holding capacity, three days) for both the varieties is established as best method of priming treatment for brinjal, capable of improving seed vigour as well as viability.

Key Words: Brinjal, Priming methods, Seed priming, Storability

Introduction

Brinjal or eggplant (*Solanum melongena* L.) is an important solanaceous crop of sub-tropics and tropics. Despite the high yielding potential of brinjal, the yield per unit area of the crop is low in India. Poor germination and low seed viability are among the serious problems limiting the production, as the use of stored seed is a common practice under tropical and subtropical conditions, leading to inadequate plant population in the field. Therefore, to improve seedling emergence by using the stored seed has been extensively studied in brinjal. Mc Donald (1999) expressed that seed priming is capable of reversing the causes of seed deterioration in storage. Mid-storage priming reduced physiological deterioration of carrot seeds in storage and ultimately showed better field emergence and yield (Dollypan and Basu, 1985).The benefits included increased germination rate, uniformity in emergence, and germination under a broader range of environments and improved seedling vigour and growth. Although, priming is acclaimed as a useful technique to invigourate the seed, yet it is also widely reported to cause detrimental effects on storage life of the subsequently dried seed (Argerich and Bradford, 1989; Tarquis and Bradford, 1992; Bruggink *et al.*, 1999). Against the popular opinion that priming reduces seed longevity, conflicting reports have been made about the viability of primed seeds during storage. In many species (e.g. *Allium cepa, Capsicum annum, Pisum sativum, Daucus carota*) improvement in seed storability after osmotic priming has been reported by Savino *et al.* (1979), Dearman *et al.* (1986) and Georghiou *et al.* (1987). In the biochemical front also contradictory arguments have been made on impact of priming on seed storability. Mc Donald (1999) reported better storability of primed seeds owing to reversal of seed deterioration due to priming but Saracco *et al.*, (1995) proposed increased sensitivity of primed seeds to deteriorative factors imposed during storage. However, the results obtained so far are few, limited and contradictory because of the varied response to treatments of cultivars and even seed lots (Bradford, 1986).

Against this background, in order to assess the storability as well as the efficacy period of the seed priming treatments, storage experiments were conducted on primed seeds of brinjal by investigating the physiological and biochemical indicators of seed vigour during storage.

Materials and Methods

Brinjal seeds of Bagyamati variety obtained for the experiment were submitted to seed priming protocols standardized by Venkatasubramanium and Umarani, (2007) *viz.,* i) hydro priming (ii) halo priming iii) osmo priming under room temperature (26+2°C) and iv) sand matrix priming at 25 ± 2°C, 100 % RH. The moisture content of primed seeds at the end of the treatment was about 35%. After the soaking period,

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Fig. 1. Effect of priming on germination percent in Bagyamati variety of brinjal

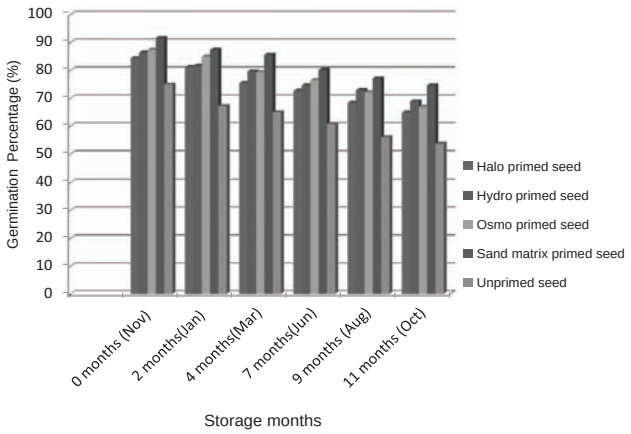
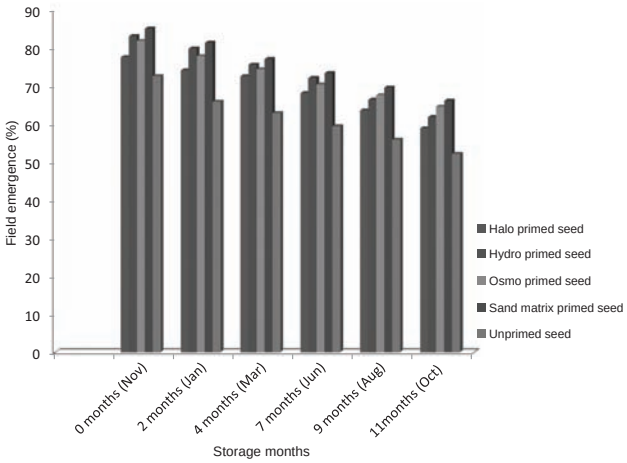


Fig. 2. Effect of priming on field emergence in Bagyamati variety of brinjal



seeds were air-dried to original moisture content under shade for three days at room temperature (26±2°C).

Storage

The seed primed with different priming solutions, after drying were packed in separate aluminium pouches along with untreated seed (control) and stored at ambient conditions (33°C and 57% RH). Seed samples from the respective treatments were drawn at bimonthly intervals and subjected to to germination test with four replicates of 100 seeds in between paper towels. The test conditions were 25±2°C and 95±5% RH. The number of normal seedlings were counted after 14 days and expressed as germination percentage.

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds used for germination}} \times 100$$

Field emergence count was taken on the 14th day after sowing in the pots and the per cent emergence was calculated taking into account the number of seedlings emerged three centimeter above the soil surface. The seeds were also analysed for electrical conductivity (Presley, 1958), dehydrogenase activity (Kittock and Law, 1968) and lipid peroxidation (Heath and Packer, 1968).

Statistical Analysis

The laboratory experiment was conducted in completely Randomized Block Design with factorial concept (FCRD). The data were statistically analysed as per the statistical methods outlined by Panse and Sukhatme, (1967).

Results and Discussion

The results revealed that significant variations were observed in all the parameters observed among the priming treatments during the storage. Brinjal seeds, showed maximum improvement in seed quality parameters due to sand matrix priming immediately after priming and even after 11 months of storage. However, the other methods of priming were also found to be better than the control. Germination percentage was 91.75, immediately after priming with the moist sand and after 11 months, it came down to 74.75 whereas, it was only 53.75 percent in control. All other priming methods *viz.*, halo priming, osmo priming and hydro priming exhibited higher germination compared to control. The field emergence of the sand matrix primed seeds was above all the other priming methods for the entire period of storage. The field emergence of the unprimed seed was inferior throughout the storage. The enzyme dehydrogenase (OD value) activity (0.117) was found to be the highest in sand matrix priming (80 WHC% 3days) while the lipid peroxidation was lowest (0.047, 0.07, 0.086, 0.106, 0.135, 0.139). The corresponding values recorded by control seeds for lipid peroxidation were 0.083, 0.16, 0.165, 0.174, 0.183, 0.186, respectively.The measure of membrane integrity i.e., electrical conductivity (dsm^{-1}), in sand matrix primed seeds, after eleven months was also lowest (0.106) compared to control (0.123).

Many studies have established that seed priming reverses seed deterioration and these beneficial effects generally occur in the meristamatic axis or the radicle tip. Sivritepe and Dourado (1994) found that humidification of aged pea seeds decreased chromosomal aberrations. During the natural process of ageing, damage in membranes and DNA leads to a loss of seed quality, this damage can be repaired during a hydration (Villiers and Edgecumbe, 1975). Surand Basu (1990) found that hydration - dehydration treatment of wheat seeds

Table 1. Effect of priming on dehydrogenase activity, electrical conductivity and lipid peroxidation in Bagyamati variety of brinjal in storage

Treatments	Dehydrogenase activity (OD Value)					
	0 months (Nov)	2 months (Jan)	4 months (Mar)	7 months (Jun)	9 months (Aug)	11 months (Oct)
Halo primed seed	0.103	0.098	0.090	0.084	0.074	0.070
Hydro primed seed	0.124	0.117	0.113	0.107	0.104	0.098
Osmo primed seed	0.124	0.116	0.112	0.097	0.094	0.092
Sand matrix primed seed	0.143	0.136	0.130	0.125	0.122	0.117
Unprimed seed	0.087	0.081	0.076	0.072	0.066	0.064
SEd±	0.003	0.002	0.003	0.002	0.002	0.002
C.D (0.05)	0.006	0.005	0.005	0.005	0.005	0.005
Electrical conductivity (dSm^{-1})						
Haloprimed seed	0.061	0.073	0.086	0.098	0.111	0.119
Hydroprimed seed	0.049	0.068	0.077	0.084	0.102	0.112
Osmo primed seed	0.060	0.075	0.084	0.097	0.111	0.118
Sand matrix primed seed	0.045	0.055	0.068	0.081	0.093	0.106
Unprimed seed	0.064	0.079	0.089	0.102	0.114	0.123
SEd±	0.001	0.002	0.001	0.001	0.001	0.001
C.D (0.05)	0.004	0.0043	0.0035	0.0033	0.004	0.0034
Lipid peroxidation (OD Value)						
Halo primed seed	0.067	0.085	0.105	0.127	0.146	0.179
Hydroprimed seed	0.059	0.072	0.086	0.112	0.125	0.146
Osmo primed seed	0.074	0.09	0.106	0.134	0.16	0.169
Sand matrix primed seed	0.047	0.07	0.086	0.106	0.135	0.139
Unprimed seed	0.083	0.160	0.165	0.174	0.183	0.186
SEd±	0.002	0.001	0.001	0.002	0.002	0.001
C.D (0.05)	0.003	0.003	0.003	0.004	0.006	0.004

enhanced germination of stored seed and reduced the production of volatile aldehydes. In onion seed, Choudhuri and Basu (1988) demonstrated that hydration - dehydration treatments effectively slowed physiological deterioration under natural (5 months) and accelerated ageing conditions with the effect dependent on the level of seed vigour. The present study also established that, seeds primed by adopting crop specific protocol for optimum duration and drying back to original moisture content ensured the efficacy of the treatment is retained in the seed during storage, besides maintaining viability of primed seeds (Venkatasubramaniam and Umarani, 2010). These results corroborates that priming benefit seeds through rectification of DNA damage, decreased chromosomal aberration, reduced electrolyte leakage etc. The problem of reduced storability of primed seeds arises only if duration of seed priming extends to an advanced stage wherein seeds enter into the Synthetic phase leading increase in DNA content (Venkatasubramaniam and Umarani, 2010). Based on the results of the present study, it is recommended that, egg plant seeds can be subjected to sand matrix priming (80% water holding capacity) for 3 days. The seeds dried back to original moisture content can be stored in aluminium foil pouches for at least eleven months without losing the efficacy of the priming treatment.

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A Note on Debatable Taxonomic Identity of *Luffa tuberosa* Roxb. (Cucurbitaceae): A Potential Wild Edible Vegetable in India

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Luffa tuberosa Roxb. (Cucurbitaceae) is of great interest to taxonomists due to its debatable taxonomic status under the genus *Luffa* and *Momordica.* The systematic study was attempted to provide evidence supporting inclusion of *L. tuberosa* Roxb. under *Momordica* as *M. tuberosa* (Roxb.) Cogn. (syn *M. cymbalaria* Hk.f.). In the present investigation comparison was made among some neglected morphological characters using 16 accessions of *Luffa* and six of *Momordica.* These characters were fruit peduncle shape, nature of pericarp, ornamentation of fruit wall, seed structure and seed dispersal mechanism.

Key Words: India, *Luffa tuberosa,* Macro- and micro-morphological characters, *Momordica,* Neglected characters, Potential vegetable, Systematic study, Wild edible vegetable

Introduction

Luffa tuberosa Roxb. is a member of family Cucurbitaceae with chromosome number 2n= 22 (Ayyangar, 1976). This perennial vine occurs wild in Indian states of Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu and also in tropical Africa mostly in dry habitat condition. This wild vegetable species is commonly known by diverse names 'Athalakkai' (Tamil), 'Karchikai' (Kannada) and 'Kadavanchi', 'Kuduhunchi' (Marathi) in India. Unripe fruits from wild are harvested and sold in the local markets of areas of occurrence. Plant propagates naturally through perennial root tubers and seeds, though the former method is more common in nature. Root tubers can be collected in the months of December-January. Plant produces flowers and fruits during October-December and seeds can be gathered in the month of December onwards.

This species has long been known for medicinal use in Indian System of Medicine especially for anti-diabetic properties similar to that of the bitter gourd. Recently it has gained attention for its potential value as neutraceutical, myocardial and cardio protective drug (Raju *et al.*, 2008). The fruits are rich in calcium, potassium, sodium and vitamin C (a known antioxidant) as compared to that of the bitter gourd (Parvathi and Kumar, 2002) and high crude fibres (6.42 g/100g) content and maleic acid (Gopalan *et al.,* 1993).

Systematic study of the species under investigation was undertaken to address debatable taxonomic position under the genus *Luffa* and *Momordica*. Comparative study using species of *Luffa* and *Momordica* based on some neglected characters like fruit peduncle shape, nature of pericarp, ornamentation of fruit wall, seed structure and seed dispersal mechanism favoured inclusion of *L. tuberosa* Roxb. into the genus *Momordica* as *M. tuberosa* (Roxb.) Cogn. (syn *M. cymbalaria* Hk.f.).

Materials and Methods

The present study was undertaken as a part of research work under the institutional project on "Systematic Study of Indian Taxa" during 2011 and 2012. The tuberous roots of *L. tuberosa* were collected from fields of Virudhunagar district, Tamil Nadu, India in the month of January 2010 and raised in earthen pots maintained in polyhouse at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi during monsoon (July 2011 and 2012). One-month-old seedlings were transplanted in the net house for detailed investigation. Accessions of taxa of *Luffa* (*L. aegyptiaca* - 6 accessions, *L. acutangula* - 3 accessions, *L. acutangula* var. *amara* - 2 accessions, *L. hermaphrodita* - 3 accessions, *L. echinata* - 1 accession, *L. tuberosa* - 1 accession) and *Momordica* (*M. charantia* var. *charantia* - 4 accessions, *M. charantia* var. *muricata* - 1 accession, *M. dioica* - 1 accession) were procured from different source localities and were simultaneously grown in the net house. Since the seeds of *M. charantia* var. *muricata* did not grow beyond seedling stage, the observations were recorded using material available in natural habitat (Sohna, Haryana) and also with herbarium specimen available in the National Herbarium of Cultivated Plants (NHCP), New Delhi. Comparison

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of characters of *L. tuberosa* was made with these taxa. Macro-morphological observations were recorded using hand lens (x10 magnification). Data on vegetative and reproductive parts were recorded from 10 plants each of the representative species. Quantitative data was recorded as an average value of each character. Measurement was done on detached plant parts. The voucher specimens and seed samples were deposited in National Herbarium of Cultivated Plants (NHCP), NBPGR, New Delhi (HS number 20630, 20631-20651).

Result and Discussion

Luffa tuberosa is of great interest to taxonomists due to its debatable taxonomic status under the genus *Luffa* and *Momordica* known with different synonyms *viz. Luffa tuberosa* Roxb., *Momordica cymbalaria* Fenzl and *M. tuberosa* (Roxb.) Cogn. On the basis of characters of endosperm haustorium and morphology of fruit that resembles *L. acutangula* (L.) Roxb. var. *amara* (Roxb.) C.B. Clarke, absence of cystoliths in leaf and absence of foliaceous bracts, it was placed under genus *Luffa* (Singh, 1964; Chakravarty, 1982). Evidences from seed coat anatomy (Singh and Dathan, 1990), seed fat characteristics (conjugated triene acid) (Azeemoddin and Rao, 1966), morpho-anatomy (Kumar *et al*., 2011), phytochemistry (Rao *et al*., 1999; 2001; Shantha *et al*., 2009) and ecogeography (Joseph, 2005) have supported its placement under the genus *Momordica*. Recent study using molecular phylogenetic data (nrDNA ITS sequences data analysis) has supported transfer of *Luffa tuberosa* to *M. tuberosa* (Roxb.) Cogn. under the genus *Momordica* (Ali *et al*., 2010).

Systematic study using morphological characters like number of tendrils, trichome structure, attachment of fruit stalk with fruit and anther lobes if free/coherent have been considered significant to delineate different taxa under Cucurbitaceae (Agdagwa and Nadukwa, 2004). Earlier investigations in support of taxonomic position of *L. tuberosa* based on evidences from morpho-anatomical characters such as cystolith in leaf, exocarp, dehiscence of fruit, seed structure and seed coat anatomy were dealt by various workers in part. While characters delineating this species on the basis of characters of peduncle, fruit wall colour at maturity, wall degeneration, nature of endocarp/aril, seed coat morphology and seed dispersal were neglected.

In the present investigation comparison was drawn among 16 accessions of *Luffa* and six of *Momordica*. Similarities in plant habit, species distribution pattern and gross morphological characters of leaf, flower, petiole and stem were noted across these two taxa. *Luffa* species are annuals with non-woody and non tuberous taproot system in comparison to that of *L. tuberosa* that is perennial, and has woody and tuberous roots similar to that of the *M. dioica* (Fig 1). All species under investigation were seed propagated unlike *L. tuberosa* and *M. charantia* var. *muricata* and *M. dioica* that were mainly propagated by tuberous roots. Tendril was trifid in *L. acutangula* and *L. aegyptiaca*, bifid *L. echinata* and tri-five fid (one developed more than rest) in *L. hermaphrodita.* Tendril was simple in *L. tuberosa* like *Momordica charantia* var. *charantia* and *M. charantia* var. *muricata*.

Fruit was green-pale green with prominent markings on pericarp of *L. aegyptiaca* and *L. hermaphrodita*, ribbed in *L. acutagula* and densely bristled in *L. echinata*. Fruit wall was dry, fibrous at maturity in all species of *Luffa*. In comparision to the above species fruit wall was ribbed-tubercled and fleshy in *M. charantia* var. *charantia*, *M. charantia* var. *muricata* and echinate in *M. dioica.*

Fig. 1 *Luffa tuberosa* **(left to right anti-clock wise): Plant growing in experimental plot; close up of male flower; branch with female flowers and tender fruits; and tuberous, woody underground root for propagation**

Fruit is a specific character in *Luffa* and in *L. tuberosa*, structure of fruit wall is neither muricate nor echinate but ribbed or angular comparable to that of *L. acutangula* var. *amara* (Chakravarty, 1882). On morphological investigation, the authors recorded pericarp of *L. tuberosa* that was similar in pattern to muricated wall of *M. charantia*. The ribs of the former species turned fibrous at maturity but in the latter species they remained soft even at maturity and degenerated on fruit dehiscence. Stopple (operculum) was present at the apical end of

fruit in all species of *Luffa* without exception but absent in all species in *Momordica*. In this respect *L. tuberosa* was morphological same as that of the *Momordica* without stopple.

Seeds were surrounded by dry papery membrane in *Luffa* but embedded in fleshy, coloured endocarp/aril in *Momordica*. The seed dispersal mechanism which is through the opening of the stopple in all species of *Luffa*. In *Momordica* degeneration of fruit wall and colour change from green to pale yellow was followed by bursting of the fruit wall. In the species under investigation the fruit wall on maturing got softened, turned yellowish-green and burst opened exposing the seeds. The fibrous nature of the mature pericarp was another important character that was exhibited by all species of *Luffa* but none of the species of *Momordica* at any stage of fruit development.

Comparison of characters in *L. tuberosa* with other taxa of *Luffa* and *Momordica* under investigation is briefed in table 1.

Conclusion

Based on comparative morphology of *Luffa tuberosa* with other taxa of *Luffa* and *Momordica* in the present investigation, the authors concluded that neglected characters such as tendril type (simple), shape of stalk at point of attachment, nature of fruit wall (non-fibrous, absence of stopple, wall degeneration at maturity), seed structure, fleshy endocarp/aril and seed dispersal mechanism were of more relevance in taxonomic study. Characters of trichome, tendril, shape of fruit stalk, fibrous/ non-fibrous nature of fruit wall at maturity and fruit wall degeneration were discussed for the first time in relevance to systematic study in this species. Thus the study supported transfer of *L. tuberosa* Roxb. into the genus *Momordica* as *M. tuberosa* (Roxb.) Cogn. (syn *M. cymbalaria* Hk. f). However, the bottleneck for detailed systematic study of this species is availability of diverse germplasm, restricted distribution, and knowledge on reproductive biology.

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Table 1. Comparison of characters of *L. tuberosa* with other species of *Luffa* and *Momordica*

Character	*L. tuberosa*	*Luffa* *	*Momordica***
Habit	Perennial; monoecious	Annual; monoecious/ dioecious	Annual, perennial; monoecious
Root	Tuberous, woody	Non-tuberous, non-woody	Tuberous, root stock, non-tuberous
Mode of propagation	Tuberous roots, seed	Root stock, seed	Tuberous roots, root stock, seed
Tendril	Simple	Two-five fid	Simple
Fruit stalk	Peduncle attenuate at apex and base	Peduncle angular to round, cuneate-obtuse	Peduncle attenuate-cuneate
Fruit characters	Dark green when young but pale yellow at maturity; prominently eight ridged	Green and pale green, wall smooth, eight ridged, echinate but never muricate	Green when young, yellow-orange, red at maturity, wall with spinose hairs, muricate but never ridged
Fruit at maturity	Fleshy, indehiscent, no stopple/ operculum, fruit bursts by wall degeneration; sponge not formed	Dry, endocarp (wall woody at maturity), fibrous, dehiscent usually by stopple; sponge formed	Wall not woody, indehiscent, endocarp not fibrous, fruit bursts by wall degeneration; sponge not formed
Seed	Few seeded; subglobose, rugose-appendaged at one end, testa without any sculpture; surrounded by fleshy endocarp	Many seeded; oblong, dry, compressed, sometimes marginate, testa smooth, endocarp not mucilaginous but membranous	Many to few seeded, smooth or corrugated/ sculpture, enclosed under the mucilaginous tissue; endocarp bright colour on maturity
Seed dispersal	Dispersal due to degeneration of fruit wall	Dispersal through stopple (operculum)	Dispersal due to degeneration of fruit wall

*Luffa** (*L. aegyptiaca*, *L. acutangula*, *L. acutangula* var. *amara*, *L. echinata*, *L. hermaphrodita*); and *Momordica*** (*M. charantia*, *M. charantia* var. *muricata*, *M. dioica*)

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Identification of Resistance Sources to Barley Yellow Rust (*Puccinia striiformis* f. sp. *hordei*) in India

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One hundred and twenty seven genotypes of barley from the various centres of the All India Coordinated Wheat & Barley Improvement Project (AICW&BIP) were evaluated by artificial inoculation in the field for two consecutive years (2009-10 and 2010-11) to identify new sources of resistance to yellow rust (*Puccinia striiformis* f. sp. *hordei*). The same set of genotypes was also evaluated against five individual races, 24, 57, G, Q and M at the seedling stage during 2009-11 in the greenhouse under controlled conditions. Based on both seedling and adult plant evaluation 10 genotypes (BHS392, DWR88, JB187, JB206, PL844, RD2786, RD2787, RD2803, RD2804 and VLB119) were found resistant across the five races of yellow rust in India. These new sources of resistance to multiple races of yellow rust would be useful in resistance breeding programme in India.

Key Words: Barley, *Puccinia striiformis* f. sp. *hordei*, Resistance, Yellow rust races

Introduction

Barley (*Hordeum vulgare* L.) is a globally important crop adapted in the marginal and stress-affected environments. Therefore it is of high importance to resource poor farmers in many developing countries. Barley is an important cereal in India and is used for variety of purposes including animal feed, human food and in industry for malting and brewing. It suffers from many diseases and amongst them the yellow rust (*Puccinia striiformis* f.sp. *hordei*) is of major importance in the main barley growing area of the north-western plains in India. The incidence of yellow rust may create havoc in susceptible varieties and results in heavy yield losses.

In conventional plant breeding, new varieties are generated from a primary adapted pool of elite germplasm. In the past decades, intensive breeding of crop varieties has further narrowed the gene pool, especially in barley. Due to limited genetic variation among modern varieties, efficient use of the genetic variation available in wild relatives of modern cultivars is therefore necessary for continued improvement. In India, barley resistance breeding has been based on sources available in landraces of indigenous or exotic origin. Yellow rust races, 24, 57, G, Q and M are more prominent in barley (Bhardwaj and Gangwar, 2012). There are very few reports on sources of resistance to yellow rust in India (Sarkar *et al*., 2003; Singh *et al*., 2004; Verma *et al*., 2008). Several varieties under cultivation have become susceptible to yellow rust because of breakdown of resistance by new virulent pathotypes. Therefore, there is a need to screen barley germplasm from diverse sources for yellow rust resistance under artificial epiphytotic conditions under different environments.

Materials and Methods

Adult Plant Resistance Test

Under the All India Co-ordinated Wheat & Barley Improvement Project (AICW&BIP), a total of 127 barley genotypes supplied by barley breeders from various centres were evaluated at seven locations (Almora, Dhaulakuan, Bajaura, Durgapura, Hisar, Ludhiana and Karnal) during 2009-10 and 2010-11 crop seasons for screening for resistance to yellow rust under artificial epiphytotic conditions (Table 1).

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Table 1. Number of barley genotypes tested for adult plant resistance

Centre	Code	Genotypes tested
Ludhiana	PL	14
Bajaura	HBL	3
Rewa	JB	6
Faizabad	NDB	10
Kanpur	K	5
Varanasi	HUB	8
Almora	VLB	6
Hisar	BH	11
Pantnagar	UPB	7
Karnal	DWR/ DWRUB	11
Shimla	BHS	5
Durgapura	RD	23

Each genotype was grown in 1 m length consisting of 10-12 plants with 30 cm distance between rows. After every 20 lines, one line of infector (mixture of susceptible genotypes- Bilara2, RD31, RS6, Jyoti and RD2035) was sown. The susceptible mixture was also grown on all four sides of test lines.

The yellow rust inoculum was received as mixtures of the most common races (24, 57, G, Q and M) from the Regional Station, Directorate of Wheat Research (DWR), Flowerdale, Shimla, and multiplied in polyhouses on susceptible genotypes at the respective centres. In the main field, susceptible infector rows were artificially injected with uredospores suspension just before early tillering stage of the crop. In addition, 3-4 sprays of water containing uredospores were also given between 45-55 days after sowing from tillering to flag leaf stage in the main field for developing rust epidemics. The rust spore sprays @5g/ litre of water were given in the evening hours. The crop was grown following the recommended agronomic practices. The data on yellow rust were recorded by combining severity (per cent leaf area covered by rust) and response (infection type). Plants were scored when the disease showed the maximum development on the infector rows. The scoring for yellow rust was done using the modified Cobb's scale (Peterson *et al*., 1948). The host response in the field was scored using 'R' to indicate resistance; 'MR' to indicate moderate resistance; 'MS' to indicate moderately susceptible and 'S' to indicate full susceptibility.

The disease severity and host response data were combined into a single value called the coefficient of infection (CI). The CI is calculated by multiplying the severity times a constant for host response: where immune=0.0, R=0.2, MR=0.4, MS=0.8, and S=1.0. The average coefficient of infection (ACI) was calculated. The genotypes showing ACI up to 1.00 for rusts at all the centres were considered highly resistant (HR). If the rust reaction at any one centre was more than zero and other centres recorded zero, the entry was subjected to reconfirmation during the second year of multi-locational screening. The lines observed as resistant were repeated for screening in subsequent years to eliminate any chance of escape.

Seedling Resistance Test

The seedling resistance test on selected resistant genotypes from field evaluation during 2008-09, was conducted at the Regional Station, DWR, Flowerdale, Shimla, using individual pathotypes (24, 57, G, Q and M) during 2009-10 and 2010-11 under controlled conditions. Eight to ten seeds per entry were sown in a row thus accommodating 10 rows in a tray. Seven to eight days old seedlings were inoculated with uredospores in talcum powder @1:500 with a spatula and placed in a moist chamber for 48 hours. Subsequently, they were placed in the glasshouse having temperatures 15-20ºC. The observations were recorded on reaction type of these seedlings against each pathotype on 14-15 day of the inoculation (Nayar *et al*., 1997).

Results and Discussion

Adult Plant Resistance

A total of 127 genotypes showing yellow rust resistance during 2008-09 and 2009-10 cropping seasons were again screened during 2010-11 in the Elite Barley Disease Screening Nursery (EBDSN) to confirm the resistance. Twenty seven genotypes (BH936, BH941, BHS387, BHS392, DWR84, DWR85, DWR87, DWR88, DWRUB73, HBL704, HBL706, HBL707, JB187, JB206, NDB1490, PL843, PL852, RD2786, RD2787, RD2798, RD2803, RD2804, UPB1008, VLB118, VLB119, VLB121, VLB122) were confirmed as resistant in adult plant stage across the locations consecutively for the next two years.

Seedling Resistance

During 2009-10, all the 127 genotypes were screened against individual races of yellow rust for seeding resistance at the Regional Station, Flowerdale, Shimla. Out of these, 31 genotypes exhibited resistance to all

Table 2. Field response of barley genotypes to stripe rust at different locations during 2009-11

Genotype	2009-2010								2010-2011							
	Almora	Bajaura	Dhaulakuan	Durgapura	Hisar	Karnal	Ludhiana	ACI	Almora	Bajaura	Dhaulakuan	Durgapura	Hisar	Karnal	Ludhiana	ACI
BH936	0	0	0	10S	0	0	0	1.4	0	0	5S	5MS	0	0	0	1.5
BH941	0	0	0	10S	0	0	0	1.4	0	0	10S	5MS	0	0	0	2.3
BHS387	0	0	0	10MS	0	0	0	1.1	0	0	20S	15MS	0	0	5MS	6.0
BHS392	0	0	5S	10S	0	0	0	2.1	0	0	0	10MS	0	0	0	1.3
DWR84	TS	0	0	10S	0	0	0	1.6	0	0	0	15S	0	0	TS	2.7
DWR85	5S	0	10S	10S	0	0	0	3.6	0	0	0	10S	0	0	5MS	2.3
DWR87	0	0	0	20S	0	0	0	2.9	0	0	10S	15S	0	0	TS	4.3
DWR88	0	0	0	0	0	0	0	0	5S	0	10S	0	0	0	0	2.5
DWRUB73	10S	0	10S	5S	0	0	0	3.6	0	10S	0	15S	0	5MS	5MS	3.8
HBL704	0	0	0	5MR	0	0	0	0.3	0	0	0	TMR	0	0	0	0.1
HBL706	0	0	0	5MR	0	0	0	0.3	0	0	0	10MR	0	0	0	0.7
HBL707	0	0	5S	5MR	0	0	0	1.0	0	0	0	5MR	TS	0	0	0.5
JB187	0	0	0	0	20S	0	0	2.9	0	0	20S	0	0	0	0	3.3
JB206	0	0	0	0	0	20S	0	2.9	0	5S	0	0	0	0	TS	0.2
NDB1490	0	0	0	0	10MS	0	0	1.1	0	0	5S	0	NG	0	0	1.0
PL843	0	0	10S	0	0	0	0	1.4	0	0	0	0	0	0	0	0.0
PL852	0	0	0	5MS	0	0	0	0.6	0	5S	5S	10S	0	0	0	2.5
RD2786	0	0	0	0	0	0	0	0	0	0	10S	0	0	0	0	1.7
RD2787	0	0	5S	0	0	0	0	0.7	0	0	20S	0	0	0	0	3.3
RD2798	0	0	0	10MS	0	0	0	1.1	0	0	40S	5MR	0	0	0	7.0
RD2803	0	0	0	0	0	0	0	0	0	0	10S	0	0	0	0	1.7
RD2804	0	0	0	0	10S	0	0	1.4	0	0	20S	0	0	0	0	3.3
UPB1008	0	0	0	5S	0	0	0	0.7	0	0	10S	10MS	0	0	0	3.0
VLB118	0	0	0	10S	0	TS	0	1.6	0	0	0	15MS	0	0	0	2.0
VLB119	0	0	0	10S	0	0	0	1.4	0	0	5S	20MS	0	0	0	3.5
VLB121	0	0	0	10S	10S	0	0	2.9	0	0	10S	20S	0	0	0	5.0
VLB122	0	0	0	10S	0	0	0	1.4		0	10S	15MS	0	0	0	3.7

S = Susceptible; MS = Moderately Susceptible; TS = Traces to Susceptible; MR = Moderately Resistant; TMR = Traces to Moderately Resistant

Table 3. Confirmation of seedling resistance by selected genotypes having adult plant resistance

Genotype	2009-2010					2010-2011				
	G (4S0)	M (1S0)	Q (5S0)	24 (0S0-1)	57 (0S0)	G (4S0)	M (1S0)	Q (5S0)	24 (0S0-1)	57 (0S0)
BHS392	R	MR	R	R	R	R	R	R	R	R
DWR88	R	R	R	R	R	R	R	R	R	R
JB187	R	R	R	R	R	R	R	R	R	R
JB206	R	R	R	R	R	R	R	R	R	R
PL844	R	R	R	R	R	R	R	R	R	R
RD2786	R	R	R	R	R	R	R	R	R	R
RD2787	R	R	R	R	R	R	R	R	R	R
RD2803	R	R	R	R	R	R	R	R	R	R
RD2804	R	R	R	R	R	R	R	R	R	R
VLB119	R	R	R	R	R	R	R	R	R	R

G, M, Q, 24, 57 = Yellow rust races

Table 4. List of identified resistant sources to stripe rust of barley

Genotype	Centre	Parentage	Origin
BHS392	IARI, Shimla	ZIGZIG/PETUNIA2// PETUNIA2	Exotic
DWR88	DWR, Karnal	DWR28/DL472	Indigenous
JB187	JNKV, Rewa	RD2508/RD2035	Indigenous
JB206	JNKV, Rewa	K560/RD2503	Indigenous
PL844	PAU, Ludhiana	TOCTE/3/CHAMICO/ TOCTE//CONGONA/4/ LIGNEE527/ GERBEL/3/BOY-B*2/ SURB//C112225.2D (30th INYT-904)	Exotic
RD2786	ARS, RAU, Durgapura	RD2634/NDB1020// K425	Indigenous
RD2787	ARS, RAU, Durgapura	RD2035/NDB1245	Indigenous
RD2803	ARS, RAU, Durgapura	P490/K560//RD2552	Indigenous
RD2804	ARS, RAU, Durgapura	RD387/BH602//RD2552	Indigenous
VLB119	VPKAS, Almora	16th HBSN-9632	Exotic

the five races and these were retested during 2010-11 for individual races under the EBDSN (Table 3). Out of the 127 genotypes tested, 10 (BHS392, DWR88, JB187, JB206, PL844, RD2786, RD2787, RD2803, RD2804, and VLB119) showed the both seedling and adult plant resistance for two years across all five races of yellow rust (Table 4).

Yellow rust in barley causes severe production lossess in northern hills, and adjoining plains. Being a crop of low input grown by marginal farmers and the disease being of multicyclic nature, chemical control is not a viable option. Therefore, resistance breeding seems to be a preferable option for the Indian barley programme. The identified sources from this study can be used as resistance donor in resistance breeding programme to minimize the losses due to yellow rust.

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Studies on Genetic Diversity in Various Qualitative and Quantitative Characters in Rice Germplasm

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One hundred and ninety five rice accessions were evaluated to study the diversity pattern among the genotypes. The genotypes were grouped under ten clusters. The distribution pattern indicated that maximum number of genotypes (28) were grouped into cluster II, followed by 27 in cluster III. The inter-cluster distance is higher than intra cluster indicating wide genetic diversity among the genotypes. The highest inter-cluster distances was observed between cluster VI and VII followed by cluster IV and VII showed wider diversity among the groups. The highest intra-cluster, distance was observed for cluster I followed by cluster IX. The accessions with IC 346231, 352813, 346237, 331640, 152036, 372020 can be used as potential donors for hybridization programme to develop variety with higher yield potential.

Key Words: Genetic Divergence, Germplasm, Rice

Introduction

Rice (*Oryza sativa* L.) is the principal staple food for more than 50% of the world's population. Rice is grown under diverse eco-geographical conditions in various tropical and sub-tropical countries, including India.

Landraces and wild species possess immense potential of most valuable gene which can be effectively utilized in the breeding programmes to develop high yielding rice varieties with quality and resistance to biotic and abiotic stresses (Saxena *et al*., 1988).

Materials and Methods

The experimental materials comprised 195 rice germplasm accessions (landraces) received from Directorate of Rice Research (DRR), Hyderabad. These genotypes were evaluated in Augumented Design during *Kharif* 2007 at Research farm, Indra Gandhi Krishi Vishwavidyala (IGKV), Raipur, Chhattishgarh (situated at $21^0$41' N latitude, 81^0 21' E longitude and at the height of 289.60 meters above the mean sea level). Twenty one days old seedlings from the nursery were transplanted. Each plot consisted of three rows of 5 m length. The spacing was maintained at 20x15 cm and the recommended practices were followed during the crop season. Five plants from middle of the row of each entry were randomly taken for observation on various 22 qualitative characters *viz.* coleoptile colour, early plant vigour, basel leaf sheath colour, leaf blade colour, leaf pubescence, flag leaf angle, ligule colour, ligule shape, collar colour, auricle colour, internode colour, panicle exertion, panicle type, stigma colour, apiculus colour, awning, hull colour, sterile lemma colour, seed coat colour, aroma and threshability. Eight quantitative characters *viz*. days to 50% flowering, plant height (cm), panicle length (cm), effective tillers per plant, no. of filled grains per plant, grain L:B ratio, 100 grain weight (g), and grain yield per plant (g). D^2 statistic was employed to measure the genetic distance between the genotypes (Mahalanobis, 1928).

Results and Discussion

A clear understanding of the extent of variability prevailing for each trait in germplasm is essential for the improvement of characters through selection. In hybridization programme, selection of genetically diverse parent is important to get wide range of recombinants. Based on qualitative descriptors, 195 accessions were classified for different characters (Table 1). Some of the qualitative characters with distinct characteristics are as follows: purple coleoptiles colour: IC325948., Purple line in basal leaf sheath colour: IC310484, IC326224, IC330649, IC331658, IC381993, IC347672., Leaf blade color with purple tips and purple margin : IC347637, IC347641, IC347643, IC346040., Purple ligule colour: IC330649, IC115503., Green collor colour: IC343382, IC347613, IC381993., Auricle color under others: IC326224, IC330036., Purple internode colour: IC330796, IC334049, IC347641., Yellow stigma colour.

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Table 1. Classification of rice germplasm accessions for qualitative characters

Characters		No. of accessions
Coleptile colour	Green	194
	Purple	1
Early plant vigour	Poor	19
	Good	105
	Very good	71
Basal leaf sheath colour	Green	164
	Purple line	7
	Light green	10
	Purple	14
Leaf blade colour	Light green	11
	Green	148
	Dark green	22
	Purple margin	9
	Purple	1
	Others	4
Leaf pubescence	Glabrous	115
	Intermediate	72
	Pubescent	8
Flag leaf angle	Erect	37
	Intermediate	133
	Horizontal	22
	Descending	3
Ligule colour	White	177
	Purple lines	16
	Purple	2
Ligule shape	Acute	9
	Cleft	186
Collar colour	Pale green	181
	Green	3
	Purple	11
Auricle colour	Pale green	184
	Purple	9
	Others	2
Internode colour	Green	124
	Light gold	64
	Purple line	4
	Purple	3
Panicle exertion	Well exerted	149
	Moderately exerted	10
	Just exerted	36
Panicle type	Compact	17
	Intermediate	135
	Open	43
Stigma colour	White	153
	Yellow	1
	Purple	41
Apiculous colour	White	20
	Straw	106
	Brown	5
	Red	33
	Red apex	9
	Purple	13
	Purple apex	8
	Others	1
Awning	Absent	169
	Short and partly awned	14
	Long and partly awned	8
	Long and fully awned	4
Hull colour	Straw	153
	Golden	2
	Brownish furrows on straw	13
	Purple furrow on straw	3
	Brown	18
	Black	6
Sterile lemma colour	Straw	171
	Red	23
	Purple	1
Seed coat colour	White	117
	Light brown	20
	Brown	6
	Red	52
Aroma	Absent	129
	Present	66
Threshability	Easy	78
	Intermediate	93
	Difficult	24

The analysis of variance (ANOVA) revealed highly significant differences among all the genotypes (Table 2) for all the characters studied of considerable amount of genetic variation. The magnitude of variation between genotypes was reflected by high mean value and range of genotype traits studied (Table 3). High genetic variability for different quantitative traits in rice has been reported earlier (Khan *et al.*, 2009; Ullah *et al.*, 2011; Seyoum *et al.*, 2012).

The results (Table 3) revealed that estimates of PCV were slightly higher than those of GCV for all traits studied. The extent of the environmental influence on traits is explained by the magnitude of the difference between PCV and GCV. Large differences between PCV and GCV values reflect high environmental influence on the expression of traits. In this study, slight difference indicated minimum environmental influence and consequently greater role of genetic factor on the expression of traits. High GCV and PCV values were observed for effective tiller per plant (26.2, 28.4), no. of grains per plant (50.97, 60.11) indicating the presence of ample variation for this character in the present material which indicate the possibility of yield improvements through selection on these traits.

Table. 2. Analysis of variation ANOVA

Source of variation	DF	50% flowering	Plant height (cm)	Effective tiller per plant	Panicle length	No. of filled grains /plant	Grain L : B ratio	100 grain weight (gm)	Grain weight per plant (g).
Replication	1	0.281	5045.476	0.5897	35.511	169824.2	4.029574	10.13308	367.54
Treatment	195	136.527**	243702.6**	11.405*	14.016*	362562.1*	0.467739**	01.11138*	141.38*
Error	195	104.226	1262.495	0.915	6.735	59230.44	0.162189	0.326485	18.277
Total	391								

Estimates of heritability and genetic advance were high for plant height (98.96, 44.2) in genotypes indicating the predominance of additive gene action for these traits, hence direct selection may be highly effective. Similar findings were reported by Thakur *et al.* (2000) and Seyoum *et al.* (2012). So these characters may be considered as important criteria for selection of the parent for hybridization program.

For quantitative characters, 195 genotypes were grouped into 10 clusters by using D^2 statistics in such a way that the genotypes within a cluster had a small or low D^2 values than those of in-between the characters. The composition of clusters has been presented in Table. 4. Cluster-II had largest number of genotypes (28) followed by cluster III (27) and cluster I (22) whereas, cluster VII had minimum number of genotypes with 12. The inter-cluster distance is higher than intra-cluster, indicating wide genetic diversity among the genotypes Table. 4. The highest inter-cluster distance varied from 4.573 to 1.965. The inter-cluster distance was observed between cluster VI and VII (4.573) (Table 5; Fig. 1). On the other hand minimum distance was observed between cluster III and IV (1.965), indicating close relationship between these clusters would not provide any good result. The greater the distance between clusters wider the genetic diversity between the genotypes. Highly divergent genotype would produce a broad spectrum of variability in the subsequent generations enabling further selection and improvement. The hybrids developed from the selected genotypes within the limit of compatibility of these clusters may produce desirable transgressive segregants or higher magnitude of heterosis. This would be useful in rice breeding programme to evolve miracle varieties with high yield potential and a similar finding was of Sarawgi and Rastogi (2000), Nayak *et al*. (2004) and Parikh *et al*. (2011).

The maximum intra cluster distance was observed for cluster I (2.127) followed by cluster IX (2.109) and cluster VI (2.079) (Table 5, Fig. 1). It was reported that genotypes within the cluster with high degree of divergence would produce more desirable breeding material for achieving

Table 3. Estimation of genetic variability, heritability, genetic advance, genetic advance in % and mean

Character	GCV	PCV	Heritability	GA	GA%	Mean
50% flowering	4.498	12.281	13.416	3.028	33.90	89.33
Plant height (cm)	216.29	217.42	98.969	712.64	44.27	160.96
Effective tiller/plant	26.23	28.43	85.14	4.38	49.80	8.73
Panicle length	7.14	12.05	35.08	2.33	8.74	26.72
No. of filled grains/plant	50.97	60.11	71.91	686.43	89.85	763.97
Grain length:width ratio	12.75	18.31	48.50	0.56	18.56	3.06
100-grain weight (g)	29.98	40.57	54.58	0.96	46.39	2.09
Grain weight/ plant (g)	51.59	58.75	77.10	14.25	93.76	15.21

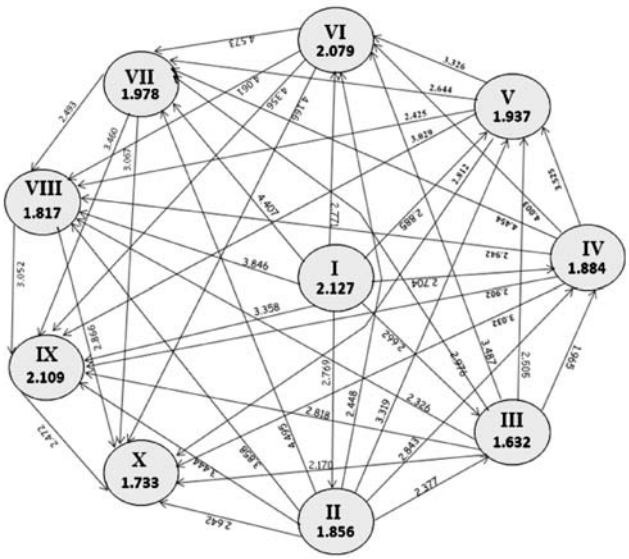

Fig. 1. Intra-and inter-cluster distance (D) in the rice germplasm

Table 4. Grouping of 195 genotypes into 10 clusters

Cluster	No. of geno-types	Genotypes with IC. No.
I	22	IC 316312, 320824, 321194, 326415, 326488, 330256, 330831, 331659, 331677, 332571, 334107, 337593, IC. No.344676, 344686, 344690, 347641, 352924, 352973, 372180, 372307, 325948, 320540.
II	28	IC 115503, 310499, 310500, 310501, 310502, 311850, 311863, 320843, 332622, 332640, 335936, 333010, 334049, 334132, 336076, 343382, 343475, 343492, 343503, 346918, 347637, 350758, 381993, 372181, 372293, 272302, 408352, 343476.
III	27	IC 306430, 311142, 311871, 316276, 316283, 320875, 330187, 331792, 332998, 334032, 334202, 340049, 340054, 340977, 343391, 346882, 351747, 342623, 342891, 372068, 360742, 363760, 381932, 381916, 381968, 381936, 396768.
IV	21	IC 310484, 310487, 311855, 311861, 330430, IC. No.332978, 336862, 340975, 343395, 346877, 346893, 346932, 347617, 347643, 347672, 340548, 340353, 342889, 350095, 350790, 320533.
V	21	IC 316273, 316277, 316378, 316279, 316311, 325162, 326224, 330836, 330649, 331658, 331785, 332975, 333018, 334111, 344735, 347638, 351697, 342864, 342622, 352764, 375774.
VI	12	IC 311864, 326477, 330699, 330807, 332019, 332040, 334046, 334321, 337616, 340072, 337002.
VII	12	IC 311856, 330226, 330251, 330260, 331646, 331640, 332031, 332043, 343982, 347613, 346039, 346234.
VIII	21	IC 152036, 310469, 319358, 319435, 330796, 330800, 330808, 331641, 332051, 333004, 352857, 352862, 352959, 353014, 346221, 346231, 346237, 350093, 350146, 352813, 352908.
IX	17	IC 325167, 326485, 330036, 330049, 330695, 332777, 334118, 334150, 334207, 337586, 351736, 351779, 356445, 340524, 342888, 372020, 382624.
X	14	IC 313549, 330055, 332049, 343546, 346843, 346865, 347669, 350292, 351738, 342618, 346040, 352836, 342864, 372845.

maximum genetic advance (Bose and Pradhan, 2005). The minimum intra-cluster distance was observed in III (1.632) followed by cluster X (1.733) and cluster VIII (1.817) indicating homogeneous nature of the genotypes with less deviation between the genotypes, therefore selection will be ineffective (Rajesh *et al*., 2010).

The cluster mean and coefficient of variation are presented in Table 6. The coefficient of variation was highest in number of filled grains/plant (423.31) followed by plant height (24.16) and lowest was recorded in grain L:B ratio (0.49). Cluster II showed highest mean value for days to 50% flowering, followed by cluster VI (95.50). Cluster X showed highest mean value for plant height (153.19) with high panicle length (30.31) and better effective tiller/plant (9.71), grain L:B ratio (2.86) and grain yield (12.62). Cluster V showed highest mean value for effective tiller/plant (12.53) with better L:B ratio (3.00) grain yield per plant (18.65). Cluster VII showed highest mean value for number of filled grains/plant (1743.92) with better panicle length (27.46), effective tiller per plant (9.97 and grain yield/plant (27.22). Cluster IX showed highest mean value for grain L:B ratio (3.91) with better panicle length (29.45). Cluster IV showed highest mean value for 100 grain weight (3.00) with better panicle length (27.86). Likewise cluster VIII showed highest mean value for grain yield/plant (28.16) with better effective tiller/plant (9.55), panicle length/ plant (27.17), number of filled grains/plant (1023.76), 100 grain weight (2.76).

The results suggest that intercrossing of genotypes from different cluster showing good mean performance may help in obtaining high yield. Inclusion of more diverse parents in hybridization is believed to increase the chances of obtaining better heterosis and give broad spectrum of variability in segregating generation.

The better genotypes can be selected for most of characters on the basis of mean performance in the cluster. The best genotypes chosen for different characters is presented in Table 7.

The genotype IC346231 of cluster VIII showed highest grain yield per plant and 100 seed weight, the IC330226 of cluster VII showed highest number of filled grain per plant with better grain yield per plant. Maximum effective tiller per plant was found in IC316273 and maximum panicle length was found in IC326485 of cluster IX.

On the basis of divergence and cluster mean it may be suggested that maximum heterosis and good recombinants could be obtained in crosses between genotypes of cluster IX, VI, VII and V in varietal improvement programme. For bringing improvement in specific traits, genotypes with IC347669 for days to 50% flowering, IC381936 for plant height, IC316273 for effective tiller per plant, IC330036 for panicle length per plant, IC330226 for number of filled grain per plant, IC356445 for grain L:B ratio, IC340353 for 100 grain weight and IC346231 for grain yield per plant could be considered in rice improvement programme (Table 7). The traits days to flowering, plant height, 1000 grain weight are the major contributors of genetic divergence.

Table 5. Average of intra-cluster (diagonal bold) and inter-cluster distance (D values) among the ten clusters in rice for characters

	I	II	III	IV	V	VI	VII	VIII	IX	X
I	2.127	2.769	2.662	2.704	2.885	2.771	4.407	3.846	3.358	3.784
II		1.856	2.377	2.843	3.319	2.448	4.495	3.858	3.444	2.642
III			1.632	1.965	2.505	3.487	2.976	2.326	2.818	2.170
IV				1.884	3.525	4.003	4.454	2.942	2.902	3.032
V					1.937	3.326	2.644	2.425	3.029	2.812
VI						2.079	4.573	4.061	4.356	4.166
VII							1.978	2.493	3.460	3.067
VIII								1.817	3.052	2.866
IX									2.109	2.472
X										1.733

Table 6. Characters mean in different clusters of rice germplasm

	1	2	3	4	5	6	7	8
I	78.32	105.57	8.16	25.08	500.68	3.23	2.21	10.54
II	96.71	125.09	7.56	25.88	399.96	2.93	1.45	5.64
III	86.78	150.35	7.21	26.50	795.78	2.70	1.96	15.66
IV	87.05	147.61	6.43	27.86	395.10	2.98	3.00	11.66
V	86.14	136.05	12.53	25.09	965.70	3.00	1.96	18.65
VI	95.50	85.09	8.45	22.68	736.67	3.07	1.86	12.07
VII	87.42	146.28	9.97	27.46	1743.92	2.87	1.55	27.22
VIII	93.14	149.60	9.55	27.17	1023.76	2.81	2.76	28.16
IX	88.41	152.33	8.91	29.45	836.65	3.91	2.01	16.66
X	94.14	153.19	9.71	30.31	855.14	2.86	1.47	12.62
Grand mean	89.27	135.87	8.68	26.67	767.89	3.02	2.05	15.24
CV%	8.23	24.16	2.39	2.63	423.31	0.49	0.73	8.36

1. Days to 50% flowering 2. Plant height (cm) 3. Effective tillers/plant 4. Panicle length (cm) 5. No. of filled grains/plant 6. Grain L:B ratio 7.100-grain weight (g) 8. Grain yield/plant (g)

Table 7. Desirable genotypes for different traits

S. No.	Character	Desirable genotype with accession number
1	Days to 50% flowering	IC 347669(109), 334321(107), 320843(107), 337002(106).
2	Plant height (cm)	IC 381936(173.7), 340054(173.6), 330049(173.0), 351779(173.0), 343982(172.0).
3	Effective tillers/plant	IC 316273(19.7), 316279(17.3), 342622(14.7), 330836(14.3), 332043(14.3).
4	Panicle length (cm)	IC 313549(32.2), 326485(33.4), 330036(33.3), 330055(31.6), 343982(31.9), 347643(32.1), 347669(32.1).
5	No. of filled grains/plant	IC 311856(1687), 316279(1716), 330226(2296), 330251(1824), 332042(2196), 343982(2042).
6	Grain L:B ratio	IC 332040(4.0), 334207(4.3), 351779(4.0), 352924(4.0), 356445(5.0), 372020(4.0), 372307(4.1), 382624(4.8).
7	100 grain weight	IC 330430(3.49), 330796(3.21), 340353(3.99), 352959(3.77), 351779(3.55), 347672(3.46), 346231(3.69), 350790(3.73), 372180(3.4).
8	Grain yield/ plant	IC No. 152036(32.76), 331640(33.06), 346231(40.79), 372020(32.60), 330226(30.35), 346237(34.77), 352813(37.73).

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Molecular Diversity in Willow Clones Selected for Commercial Plantation

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Twenty-five promising willow clones from six countries were selected after nursery screening. Molecular diversity among the clones was estimated using 16 RAPD and 14 SSR primers. A total of 363 markers were generated and out of which 351 were showing high (96.7%) rate of polymorphism. Out of 30, 18 primers showed 100% polymorphism. RAPD markers widely scored over SSR markers in polymorphic information content. UPGMA dendrogram based on both RAPD and SSR markers resolved into four major clusters. The similarity coefficient among 25 clones of willow ranged from 0.71 to 0.86. Maximum similarity (86%) was observed between 795 and PN-721 and SI-63-007 and V-99. Thus, these genotypes showed maximum degree of similarity in their genetic makeup. However, minimum values were observed between SE-69-002 and 84/11 (0.71) followed by SE-69-002 and 17-93-A (0.72). RAPD and SSR analysis proved helpful for estimating the magnitude of genetic diversity at molecular level. Clustering further indicated that the geographic distribution may not be the true index of genetic diversity in willow clones. On the basis of banding pattern RAPDs and SSRs were effectively used for molecular characterization of willow clones used in this study.

Key Words: Characterization, Molecular diversity, RAPD, SSR, Willow

Introduction

The introduction of DNA markers provided a good discriminatory system, independent of environmental conditions. The random amplified polymorphic DNA (RAPD) technique has been applied in several studies to successfully distinguish between tree species or clones. Nowadays, simple sequence repeat (SSR) have been proven to be very suitable markers for cultivar identification in olive as they are transferable, highly polymorphic and co-dominant markers (Carriero *et al*., 2002; Cipriani *et al*., 2002). These methods have no requirement for probes or radioactive material, and can, therefore, be applied immediately. They have proven particularly useful in identification of clones and in characterization of genetic diversity. Over the past decade, a number of molecular techniques have been developed that can provide information on genetic diversity and genetic relationships. Although, there are potentially many techniques to choose from, the choice becomes more restricted when few prior molecular studies have been carried out, as is the case for willow (Lin *et al*., 1994a; Lin *et al*., 1994b). Molecular markers play an essential role today in all aspects of plant breeding, ranging from the identification of genes responsible for marker-aided selection to quantitative trait loci studies.

Increasing concerns over global climate change have led to very practical emphasis in exploiting plants as sources of renewable energy for heat. High yielding perennial biomass crops under short rotation forestry are projected to make important contributions to the future energy mix due to low inputs and, thus, favorable energy and greenhouse gas balances associated with their production systems. Willows, grown as short rotation coppice, are among the most advanced biomass crops in temperate and sub-tropical regions because of their potential for high yields in short cultivation cycles, ease of vegetative propagation and ability to resprout after multiple harvests. A major advantage offered by willow, however, is the large genetic diversity that is present in the genus, which, together with the proven characteristics *i.e.* easy hybridization, provides a rich resource for crop improvement.

The genus *Salix* comprises about 350–500 species worldwide. Some of the tree species have been cultivated for a variety of end-uses *viz.* baskets, cricket bats, handles, furniture's etc. The genus is distributed over wide ecological and climatic zones ranging easterly from North America to China, excluding Australasia (Skvortsov, 1999). *Salix* tree improvement projects are vigorously taken up in many countries in the world. Research mission include the study of willow taxonomy,

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morphology, physiology, cultivation practices, advanced breeding, molecular fingerprinting and genome mapping (Kuzovkina *et al*., 2008). Wide range of collection, evaluation and multiplication of superior willow clones and production of interspecific hybrids are necessary for increasing biomass production (Zsuffa and Mosseler, 1986). Under such evaluation and distribution study, proper identification of species and clones is essential. Efforts are currently underway to breed willows for biomass production, wood production, soil conservation, energy plantation and myriad wood products for cricket bat (Singh and, Huse, 2004; Singh *et al*., 2004). Around 200 clones/strains covering twenty-five species from twenty countries were procured and subjected for repeated nursery screening at university campus. Field trials were undertaken in order to assess suitability of clones and juvenile mature correlation in different agro-climatic regions.

Molecular markers specially RAPD and SSR are being applied to a greater extent in forest trees to study genetic diversity and contributes about 25 per cent and 19 per cent respectively of the total molecular markers used in forest biotech activities (FAO, 2004). Molecular markers assess variations in the nucleotide sequence of DNA of different individuals. Molecular markers are numerous and therefore a large genome can be easily assayed for existence of any variation, such genetic markers are easy to score. Molecular markers were earlier used for studying genetic relationships among 154 genotypes, including 50 species, held within the UK National Willow Collection (Trybush *et al.,* 2008). Use of molecular markers therefore provides an objective assessment of genetic diversity in a plant species and enables unequivocal identification of elite genotypes.

In *Salix* various molecular markers *viz.,* RAPD, SSR and AFLP have been used to assess genetic diversity for germplasm characterization (Barker *et al*., 1993; Barker *et al*., 2003). However, the clones selected in the present study were not investigated earlier for molecular characterization. The present investigation was undertaken with the objective of estimation of genetic diversity using RAPD and SSR markers.

Materials and Methods

Plant Material

A set of twenty five willow clones (Table 1) of genus *Salix* were procured from five countries namely, UK, Italy, New Zealand, Croatia and Turkey. These clones were selected based on their performance from nursery screening experiments done on the basis of plant height and collar diameter.

Table 1. Original identity with scientific name and source country

Treat-ment	Original Identity	Scientific name	Source country (procured from)
T1	PN-733	*Salix nigra* Marsh.	New Zealand
T2	17-93-A	*S. alba* Linn.	New Zealand
T3	SE-63-016	*S. jessonensis* Seeman.	Italy
T4	PN-722	*S.matsudana* Kiodz.	New Zealand
T5	212/03	*S.matsudana* X *S. caprea*	UK
T6	84/11	*S.alba* Linn.	Turkey
T7	MB-368	*S.alba* Linn.	Croatia
T8	PN-227	*S.matsudana* Koidz.	New Zealand
T9	SI-64-017	*S.alba* Linn.	Italy
T10	NZ-1002	S.matsudana X S.alba	New Zealand
T11	795	S.matsudana X S.alba	UK
T12	SI-63-007	*S.alba* Linn.	Italy
T13	V-99	Salix X rubens	Croatia
T14	NZ-1179	S.matsudana X S.alba	UK
T15	PN-721	S.matsudana X S.alba	New Zealand.
T16	131/25	S. alba X S. babylonica	UK
T17	799	S.matsudana X S.alba	UK
T18	PN-731	*S.nigra* Marsh.	New Zealand
T19	NZ-1040	S.matsudana X S.alba	New Zealand
T20	NZ-1140	S.matsudana X S.alba	UK
T21	SE-75-001	*S.matsudana* Koidz.	Italy
T22	NZ-1130	S.matsudanaa X S.alba	New Zealand
T23	V-311	*S.matsudana* Koidz.	Italy
T24	SE-69-002	*S.matsudana* Koidz.	Italy
T25	Kashmir Willow	*S.alba cv. Coerulea* (Smith) Dumort.	UK

Genomic DNA Extraction

Young leaf tissue was powdered in liquid nitrogen. The powder was either stored at -40^{0}C or used for DNA isolation immediately. Total genomic DNA was isolated using the CTAB (Cetyle- trimethyl ammonium bromide) method advocated by Doyle and Doyle (1987) with slight modification made in buffer concentrations. The quality of DNA was tested on 0.8 per cent agarose gel (Genei Banglore, Banglore India) and quantification was done using Perkin Elmer UV/VIS spectrophotometer and diluted to 5ng/µl for further PCR (Polymerase Chain Reaction) amplification using CR Corbett thermocycler.

PCR Amplification and Electrophoresis

RAPD amplification

Sixteen out of 20 initially screened decamer primers (Genei Banglore, Banglore India) were used for the current study (Table 2). DNA was amplified by PCR amplification reaction. The 25µl of reaction mixture contained 20ng of DNA, 0.75 units of Taq DNA polymerase (Genei Bangalore, Bangalore India), 2.5µl of 10X Taq buffer (50mM MgCl2, 10mM Tris-Cl), 1.25µl of pooled dNTP's (2.5mM each) and 10ng of primer (Genei Bangalore, Bangalore India). PCR conditions used for RAPD amplification included initial denaturation for 3minutes at 94^0C followed by 45 cycles of amplification (denaturation at 92^0C for 45 seconds, annealing of primer at 36^0C for 1 minute and primer amplification at 72^0C for 2 min) and final extension at 72^0C for 10 minutes.

SSR amplification

A set of fouteen SSR primers (Genei Bangalore, Bangalore India) were selected (Barker *et al.,* 2003) for PCR analysis based upon their performance and reproducibility, among them 10 primers had given distinct polymorphism (Table 4). PCR mixture of 25µl contained 20ng of genomic DNA template, 0.6 unit of Taq DNA polymerase (Genei Bangalore, Bangalore India), 2.5µl of 10X Taq buffer (50mM MgCl2, 10mM Tris-Cl), 1.25µl of pooled dNTP's (2.5mM), 20ng of each reverse and forward primer (Genei Bangalore, Bangalore India). PCR conditions used for SSR amplification were initial denaturation at 94^0C for 3 minutes followed by 35 cycles of amplification (denaturation at 94^0C for 45 sec, annealing of primer ranged from 55-60^0C for 1 minute and primer amplification at 72^0C for 2 min) and final extension at 72^0C for 20 minutes.

Electrophoresis

The amplified products as developed by the primers were separated on agarose 1.4 % and 2.5 % gel in 1X TAE, stained in ethidium bromide respectively for RAPD and SSR through electrophoresis and photographed in Alpha Imager Gel Documentation System. 1kb and 100bp DNA mass ladder (Genei Bangalore, Bangalore India) were used as molecular weight markers in first well of respective gel.

Data Analysis

The RAPD and SSR marker amplification profile of 25 clones was used to estimate genetic diversity/relatedness based on number of shared amplified bands. The presence or absence of a particular amplification product was used as an index of genetic diversity/relatedness. The similarity matrix value based on Jaccard's coefficient of similarity (Simqual function) was used to generate dendrogram.

Table 2. Total numbers of amplified and polymorphic fragments generated by PCR using RAPD primers along with their nucleotide sequences

S. No	Primer name	Base sequences (5'-3')	Total no. of scorable bands (y)	Total no. of polymorphic bands(x)	Total no. of monomorphic bands	Polymorphism (%) $\frac{x}{y}X100$	Size range of amplified products (bp)
1	OPJ-05	CTCCA-TGGGG	15	15	0	100	300-3500
2	OPO-03	CTGT-TGCTAC	25	25	0	100	350-2250
3	OPO-04	AAGTC-CGCTC	21	21	0	100	350-3500
4	OPO-06	CCACG-GGAAG	8	7	1	87.5	300-1500
5	OPO-07	CAGCA-CTGAC	24	24	0	100	300-1750
6	OPO-12	CAGTG-CTGTG	22	22	0	100	250-2000
7	OPO-15	TGGC-GTCCTT	19	19	0	100	400-2250
8	OPO-19	GGTGC-ACGTT	20	20	0	100	300-1750
9	OPO-16	TCGGC-GGTTC	10	10	0	100	200-2500
10	OPA-17	GACCG-CTTGT	24	24	0	100	300-2000
11	OPA-18	AGGTGACCGT	29	29	0	100	150-2500
12	OPA-19	CAAACGTCGG	24	24	0	100	250-2000
13	OPA-20	GTTGCGATCC	18	18	0	100	300-2500
14	DECA-05	CCAAGGGGGC	32	32	0	100	200-3800
15	DECA-07	CCGCCCGGAT	28	28	0	100	200-3500
16	DECA-12	CTTGCCCACG	20	20	0	100	200-2000
Total			339	338	1	99.7	150-3800

Clustering was done by UPGMA using SHAN module of NTSYSpc. Version 2.02e (Rohlf, 1998).

Results and Discussion

RAPD analysis

Out of 16 RAPD primers (Table 2) selected for study, three primers showed uniqueness by producing specific bands for three genotypes specific bands (Table 3). A total of 339 amplified products were obtained (using alpha imager software tool) with an average of 21.2 amplicons/primer, out of which 338 were polymorphic showing high degree of polymorphism (99.4%). The number of RAPD markers generated per primer varied from 8 to 32 because of the primer sequence and due to individual genotype (Table 2). This is consistent with the studies carried out in willow and poplar clones (Barker et al., 1993). Earlier Castiglione *et al.* (1993) performed RAPD analysis on 32 clones belonging to different species of genus Populus. Four primers out of eighteen tested were selected on the basis of number and frequency of the polymorphism produced. The results showed that the RAPD analysis allowed discriminating all the clones. DNA amplification pattern as detected by four RAPD primers used for in the present study has been presented in figures 1 (a) and 1 (b).

Primers namely OPO-07, OPO - 16 [Fig 1(a)], and OPO-19, produced unique bands present only in genotypes 17-93-A, 212/03, and SI-64-017 respectively. This information could be used for characterizing particular genotypes (Table 3). Similar trends were obtained using allozyme loci, nuclear DNA Restriction Fragment Length Polymorphism (RFLP's) and Random Amplified Polymorphic DNA (RAPD's) in 130 trembling aspen (*Populus tremuloides* Michx) and 105 bigtooth aspen (*P. grandidentata* Michx) trees (Liu and Furnier, 1993). RAPD proved to be a very powerful tool for fingerprinting aspen individuals. Earlier working on the same lines Su *et al.* (1995) worked out the variation in the genomes of 16 clones in two *Salix* species (*S. viminalis* and *S. dasyclados*) using 12 random decamer primers. The amplified DNA fragments recorded 5-18 clear bands ranging from 220-2036 bp in length in case of S. dasyclados Wimm by the primer CHL1, while all the 16 clones could be identified by the primer Deca-4 which produced large number of polymorphic bands. Similarily Sanchez *et al.* (1998) used RAPD markers to distinguish 25 poplar clones, namely, five of *Populus nigra*, five of *P. deltodies*, five of *P. alba*, five of *P. tremula*, one of *P. trichocarpa*, three of *P.* x *canescens* and one clone (*P. tremula* x *P. alba* "Bolleana") *i.e. Platero*

Table 3. Informative RAPD and SSR markers specific for a particular clone

Primer	Approximate size of DNA band	Clone
OPO-4	300bp	17-93-A
OPO-19	1500bp	212/03
OPO-16	400bp	SI-64-017
OPO-16	1750bp	SI-64-017
SB-80	200bp	SE-75-001

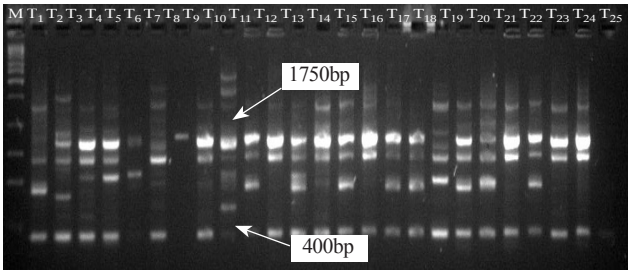

Fig. 1(a). RAPD fingerprints of willow clones using Primer OPO- 16

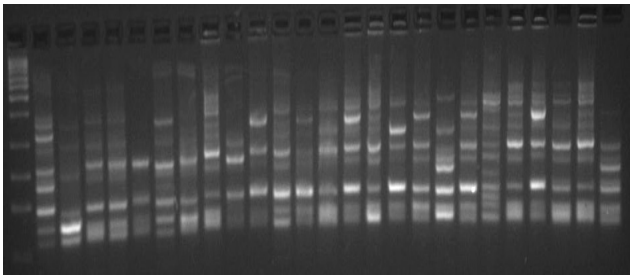

Fig. 1 (b). RAPD fingerprints of willow clones using Primer OPO- 04

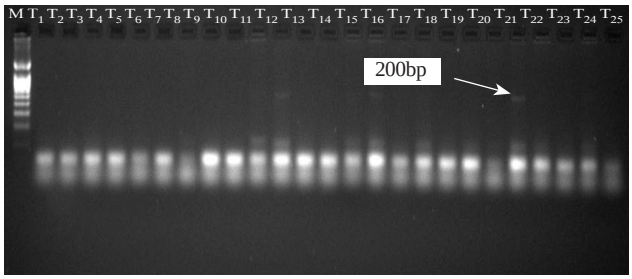

Fig. 2(a). SSR fingerprints of willow clones using Primer SB-80

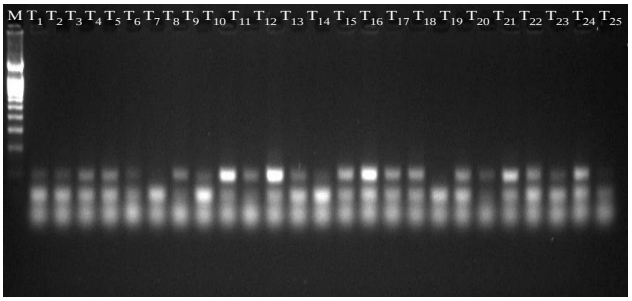

Fig. 2 (b). SSR fingerprints of willow clones using Primer SB-100

SSR Analysis

Ten out of 14 SSR primers selected for study (Table 4) showed polymorphism and selected for further analysis as given in Fig. 2 (a) and Fig.2 (b). Out of these, one primer (SB-80) exhibited unique band (Table 3) present only in clone SE-75-001 from Italy [Fig. 2(a)]. A total of 34 amplified products were obtained with an average of 3.4 amplicons/primer, out of which 27 (79.4%) bands were polymorphic. The number of SSR markers generated per primer varied from 2 to 10 because of the primer sequence and due to individual genotype (Table 4). This is consistent with the studies carried out in willow clones by Singh and Huse (2004) and Barker *et al.* (2003), on Willows by Tuskan *et al.* (2004) on *Populus trichocarpa* and for the use of poplar micro-satellites DNA markers in willow by Rajora and Rehman (2005). Similarly spatial genetic structure and diversity of alpine *Salix* species was assessed by Christoph *et al*. (2007) using microsatellite (Simple Sequence Repeat) analysis. SSR analysis, based on three loci and 16 alleles, revealed 24 different genotypes and a proportion of distinguishable genotypes of 0·18.

Cluster Analysis

UPGMA dendrogram based on both RAPD and SSR markers resolved into four major clusters (Fig. 3). The similarity coefficient among 25 clones of willow ranged from 0.71 to 0.86 (Table 5) indicating that there was more similarity among the set of clones selected. A critical perusal of dendrogram reveals that the distribution of various genotypes into clusters and within cluster was somewhat random. This is in agreement with studies carried out by Niak and Dandin (2005) on *Melia* and by Trybush *et al*. (2008) on willows. Cluster one comprised of only four genotypes *viz.* PN-733, 17-93-A, SE-63-016 and PN-722 showing 78 per cent similarity with rest of the genotypes taken for study. Cluster II comprises five genotypes viz. 212/03, 84/11, SI-64-017, Kashmir willow and MB-368 showing 79 per cent similarity among rest of the clones.

Cluster III comprises of two clones *viz.* PN 227 and PN 731 both from New Zealand showing similarity of 80 per cent with cluster IV. Cluster IV a major cluster having 14 genotypes were grouped in to four sub clusters. Sub cluster one having genotypes NZ-1002, NZ-1040 and 799 showed 85% similarity among themselves and 83% with rest of the clones of cluster IV. Second sub cluster comprises four genotypes *viz.* SI-63-007, V-99, V-311 and SE-69-002 of which SI-63-007 and V-99 showed maximum similarity between themselves (86%). Genotypes 795, PN-721, SE-75-001, 131/25, NZ-1130

Table 4. Total number of amplified and polymorphic fragments generated by PCR using SSR primers along with their nucleotide sequence

Primer name	Sequences	Total no. ofscorable bands (y)	Total no. of polymorphic bands (x)	Total no. of monomorphic bands	Polymorphism (%) $\frac{x}{y}X100$	Size range of products (bp)
SB-38	FP-CCACTTGAGGAGTGTAAGGAT RP-CTTAAATGTAAAACTGAATCT	10	9	1	90	100-400
SB-194	FP-TGTGAGATAAGATTTGTCGGT RP-CCATAAATAAAAAACGTGAAC	2	1	1	50	100-200
SB-85	FP-CTCAGCAACTTAATCCAACTA RP-GTTTGTTAGGGGAGGTAAGAA	3	3	0	100	100-150
SB-80	FP-TAATGGAGTTCACAGTCCTCC RP-ATACAGAGCCCATTTCATCAC	4	4	0	100	100-250
SB-196	FP-CTGTTTCCTGCCACTATTACC RP-TATAATCTGTCTCCTTTTGGC	2	1	1	50	100-200
SB-201	FP-CCTCTTTTTCTATTGTGGTCT RP-GGCATGTATTTTTACTCCAAC	2	1	1	50	100-300
SB-210	FP-TATAAAGACAAATACCTGGGG RP-CATCAAAGACTGCTAGAAAGG	3	2	1	66	100-200
SB-100	FP-ATGTCATTCAGGTTTGTTTTC RP-ATGGTTTAACTTGTTACTGTA	3	2	1	66	100-300
SB-88	FP-TATTGCTTTGATGGCGACTGC RP-CAGCAACGGAAATAGCAACAG	2	1	1	50	100-350
PHTR-3	FP-ATTTGCATCCAGTCTTCAGTAATT RP-CTCAAAGAAGTGCATAGAGATTTCAT	3	3	0	100	100-250
	Total	34	27	7	79.4	100-400

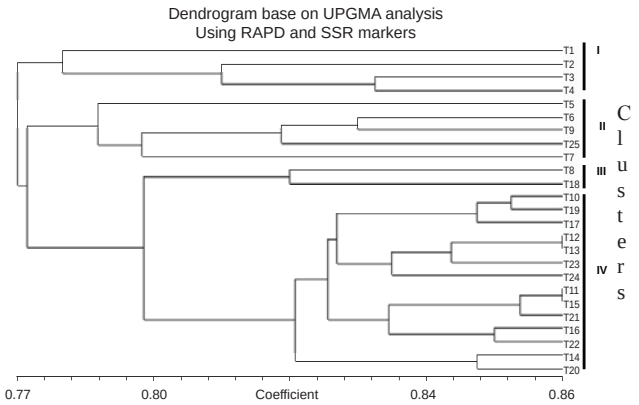

Fig. 3. Dendrogram based on UPGMA analysis of 25 clones of willow using RAPD and SSR markers

forms sub-cluster three of which 795 and PN-721 showed highest similarity in their genetic makeup. Sub-cluster four had only two genotypes *i e.* NZ-1179 and NZ-1140 showing 85 per cent similarity.

The similarity coefficient values were highest (0.86) between the genotypes 795 and PN-721, SI-63-007 and V-99. Thus these genotypes showed maximum degree of similarity in their genetic makeup. However, the minimum values of similarity coefficients were observed between SE-69-002and 84/11 (0.71) followed by SE-69-002 and 17-93-A (0.72). These genotypes are highly diverse and should be used for hybridization and improvement programmes.

Cluster I, the most diverse cluster having four species and retains four genotypes namely PN-733 (*S. nigra*), 17-93-A (*S. alba*), SE63-016 (*S. jessoensis*), PN-722 (*S. matsudana*). II and all these clones belong to the species *Salix alba* except clone 212/03 and it is the cross between *S. matsudana* and *S. caprea.* It shows clear-cut distinction from rest of the four clones of the cluster and this hybrid has all together different genetic makeup. Cluster III had two clones viz. PN-227 (*S. matsudana*) and PN- 731 (*S. nigra*) which were developed in New Zealand on the basis of plant material procured from China and U.S.A. (Kraayenoord *et al*., 1995). As regard the cluster IV, there are fourteen clones and mostly these clones were the hybrids of *S. matsudana* and *S. alba* or *S. alba* and *S. matsudana* alone. The *S. babylonica* is

Table 5. Similarity coefficient values of RAPD and SSR data using Jaccard's Similarity Correlation Coefficient

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	T_{17}	T_{18}	T_{19}	T_{20}	T_{21}	T_{22}	T_{23}	T_{24}	T_{25}
T_1	1																								
T_2	.77	1																							
T_3	.76	.80	1																						
T_4	.77	.79	.82	1																					
T_5	.77	.74	.79	.78	1																				
T_6	77	.77	.77	.77	.76	1																			
T_7	.73	.75	.74	.75	.77	.79	1																		
T_8	.77	.78	.81	.82	.78	.77	.77	1																	
T_9	.75	.75	.78	.79	.78	.82	.79	.78	1																
T_{10}	.76	.77	.81	.80	.79	.76	.82	.79	.82	1															
T_{11}	.76	.78	.81	.81	.78	.77	.81	.81	.80	.84	1														
T_{12}	.77	.80	.82	.80	.79	.78	.80	.82	.82	.85	.86	1													
T_{13}	.77	.77	.81	.79	.77	.73	.77	.83	.77	.82	.85	.86	1												
T_{14}	.75	.73	.77	.76	.76	.74	.75	.79	.79	.82	.82	.81	.81	1											
T_{15}	.76	.74	.79	.77	.78	.75	.79	.79	.75	.81	.86	.80	.82	.82	1										
T_{16}	.77	.75.	.81	.78	.80	.77	.78	.78	.79	.85	.84	.85	.83	.83	.82	1									
T_{17}	.77	.78	.81	.77	.79	.78	.76	.80	.80	.85	.83	.83	.83	.80	.82	.83	1								
T_{18}	.74	.75	.78	.78	.74	.75	.77	.81	.75	.79	.81	.82	.80	.79	.78	.80	.80	1							
T_{19}	.77	.78	.80	.78	.75	.75	.78	.80	.79	.86	.84	.82	.83	.81	.82	.81	.85	.80	1						
T_{20}	.76	.77	.77	.76	.77	.75	.77	.78	.80	.81	.82	.81	.80	.85	.83	.83	.83	.80	.84	1					
T_{21}	.78	.76	.79	.79	.77	.74	.77	.82	.76	.84	.86	.81	.85	.81	.87	.82	.81	.78	.85	.82	1				
T_{22}	.75	.75	.78	.75	.78	.75	.78	.77	.77	.81	.86	.80	.83	.81	.83	.85	.80	.80	.79	.82	.84	1			
T_{23}	.78	.76	.78	.77	.76	.77	.79	.81	.77	.83	.85	.84	.85	.82	.83	.84	.84	.80	.83	.84	.84	.82	1		
T_{24}	.75	.72	.76	.75	.77	.71	.76	.80	.75	.81	.82	.82	.84	.79	.83	.81	.80	.77	.82	.80	.85	.81	.83	1	
T_{25}	.77	.78	.75	.77	.80	.81	.81	.80	.82	.82	.82	.82	.80	.78	.77	.79	.82	.79	.81	.78	.77	.77	.82	.76	1

closely allied species of *S. matsudana* on the basis of phylogenetic study (Skvortsov, 1999).

Sub-cluster one of Cluster IV had three clones and all are hybrids *i.e.* cross between *S. matsudana* and *S. alba*. Similarly, sub-cluster three had five hybrids of *S. matsudana* and *S. alba* or *S. alba* and *S. babylonica* whereas sub cluster four had only two clones and both are hybrids between *S. matsudana* X *S. alba* which were developed in New Zealand (Stott, 1984). Clone 795 and PN-721 are the hybrids of *S. matsudana* and *S. alba* developed in different countries *i.e.* China and New Zealand found most similar one.

Conclusion

The extent of polymorphism indicated was marginally higher in RAPD compared to the SSR markers and hence, the former is more informative that the later. The RAPD profile usually represent available portion of the genome while the SSR markers represent microsatellite rich regions. The result, thus, highlights the utility of two different marker systems in providing great information on the genetic structure of *Salix* clones. Markers used were also helpful in characterizing willow clones. The clustering pattern exhibited that the geographic distribution does not provide true index of genetic diversity in willow clones. This is because of the fact that willow resources have been freely exchanged all over the world and used for willow breeding for production of vigorous clones.

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Molecular Characterization of Sorghum Germplasm for Drought Tolerance Exhibiting Stay-green Trait

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The best characterized form of drought tolerance in sorghum during crop growth is the non-senescence or "stay-green" trait. To assess the genetic diversity available in sorghum germplasm for drought tolerance, 16 stay-green specific simple sequence repeat markers (SSR) were used of which 10 were polymorphic. A total of 47 scorable alleles were generated by these 10 primers of which 44 were polymorphic (93.6%). The number of alleles produced by different primers ranged from three to seven with an average of 4.7 alleles per primer**.** Similarity coefficients based on 10 polymorphic SSR markers ranged from 0.205 to 0.864. The dendrogram grouped the sorghum accessions into 11 major clusters and categorized the drought-resistant and drought-susceptible genotypes into separate clusters. Most of the genotypes in the cluster I and IV had better stay-green score ranging between 1.80 to 2.5. The study revealed that the presence of genetic diversity is high among the accessions and the stay-green specific primers sorted the genotypes based on their level of drought tolerance.

Key Words: Cluster, Drought tolerance, Polymorphism, Simple sequence repeats, Stay-green

Introduction

Drought response in sorghum has been characterized at both pre-flowering and post-flowering stages. Drought stress during the post-flowering stage needs serious consideration because the negative impact of post-flowering drought on yield can be very drastic. Yield reduction can result from the loss of yield associated with premature plant death, stalk rot, lodging and reduced seed size of post-flowering drought-susceptible cultivars. Post-flowering drought adaptation in sorghum is associated with the stay-green phenotype. Genotypes with resistance to post-flowering stress retain their leaves in an active photosynthetic state when subjected to water stress conditions during the grain-filling period. Such genotypes are described as possessing the stay-green trait which makes the plant resist premature senescence, retain green leaves, fill grain normally and resist lodging under conditions of post-flowering drought stress (Rosenow *et al*., 1977).

As the stay-green is expressed only in those tests in which terminal stress occurred, neither the efficiency nor the reliability of selection is high when only conventional breeding approaches are used for selection of this trait. In that scenario, the use of molecular markers could help improve selection efficiency for drought resistance. SSR markers have been used as a successful tool in genotyping and studying the genetic diversity of many plant species because of their reproducibility, multiallelic nature, co-dominant inheritance, relative abundance and good genome coverage as compared to other DNA markers. Stay-green trait and the SSR markers linked to them have been identified in sorghum (Crasta *et al.*, 1999; Tao *et al.*, 2000; Xu *et al.*, 2000; Kebede *et al.*, 2001; Subudhi *et al.*, 2002) which would facilitate a more efficient selection of genotypes for drought resistance in order to gain stable and optimal yield under drought conditions. Thus, the study was undertaken to assess the extent of genetic diversity available in sorghum for drought resistance by using stay-green specific, simple sequence repeat markers, Tamil Nadu.

Material and Methods

The experimental material for this study comprised 100 accessions of sorghum, which were adapted to different agroclimatic zones of Tamil Nadu (Table 1). The seeds were obtained from germplasm collection maintained at Millet Breeding Station, Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

Visual Rating of Stay-green

The stay-green trait was estimated visually on plot basis, on a scale of 1 to 5, based on the degree of leaf and

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Table 1. The 100 sorghum genotypes used in the study

Entry	Source	Entry	Source
B 35	NRCS, Hyderabad	AS 5779	Coimbatore
CO 26	Coimbatore	AS 5787	Coimbatore
AS 1	Palani	AS 6002	Perundurai
AS 5058	Gudiyatham	AS 6184	Kangeyam
AS 5055	Dindigul	AS 7759	Omalur
AS 321	Manapparai	AS 6329	Dharapuram
AS 557	Dindigul	AS 8747	Coimbatore
AS 1038	Cumbam	AS 6616	Kangeyam
AS 1041	Gobi	K 3	Kovilpatti
MS 73	Trichy	AS 8735	Voyalpad
CO 21	Coimbatore	LS 120	Cutralam
CO 22	Coimbatore	LS 161	Rajapalayam
CO 25	Coimbatore	AS 9772	Erode
M 35-1	NRCS, Hyderabad	AS 8007	Palayamkottai
AS 391	Salem	AS 5573	Dindigul
AS 668	Thirunelveli	AS 9841	Thirunelveli
AS 817	Coimbatore	AS 9857	Omalur
AS 5052	Karur	AS 9912	Salem
AS 3776	Coimbatore	AS 9916	Thirunelveli
LOCAL 3	Coimbatore	MS 7819	Salem
TENKASI 1	Tenkasi	MS 7826	Vellore
AS 2059	Coimbatore	MS 7837	Vellore
AS 2699	Coimbatore	MS 7818	Chittirapatti
AS 2752	Coimbatore	MS 7738	Omalur
AS 5078	Periyakulam	MS 5133	Perambalur
AS 3283	Kovilpatti	MS 5826	Thirumangalam
AS 3850	Madurai	IS 5379	Coimbatore
AS 5057	Vellore	MS 7703	Trichengode
AS 7742	Omalur	MS 7725	Salem
AS 3998	Thirunelveli	MS 7728	Salem
AS 9447	Thirunelveli	MS 7843	Vellore
AS 8021	Thirumangalam	MS 7863	Ariyalur
AS 4107	Perundurai	MS 7804	Vellore
AS 4153	Kovilpatti	MS 7806	Vellore
AS 4226	Gobi	Murunga-patti local	Murungapatti
AS 4242	Erode	Uppam cholam	Coimbatore
AS 4243	Erode	VS 1564	Vamban
AS 4265	Kovilpatti	AS 900	Manappari
AS 4289	Manamadurai	AS 3284	Kovilpatti
AS 4294	Karur	AS 3285	Coimbatore
AS 4567	Pollachi	AS 3289	Coimbatore
AS 4599	Kovilpatti	AS 9098	Thirunelveli
AS 4641	Pollachi	AS 9783	Dharapuram
AS 4647	Kovilpatti	MS 7861	Trichy
AS 4654	Ramnad	MS 7862	Trichy
AS 8038	Thirumangalam	MS 7816	Thiruman-galam
AS 4959	Kangeyam	VS 1560	Vamban
AS 8262	Thirumangalam	CO 24	Coimbatore
AS 8444	Gobi	CO 1	Coimbatore
AS 5773	Dindigul	ACM 6	Madurai

plant death at physiological maturity in the field under post-flowering drought stress as described by Wanous *et al*. (1991). A rating of 1 indicated essentially no leaf death, while a rating of 5 corresponded to complete plant death (leaves and stem).

Genotyping

The genomic DNA was isolated from 100 sorghum genotypes according to Gawel and Jarret (1991) and diluted to 25ng/l. Its concentration was estimated through Fluorometer (Model DYNA Quant 200, Hoefer, California, USA) and agarose gel electrophoresis. Sixteen stay-green specific SSR markers described by Crasta *et al*. (1999), Xu *et al*. (2000) and Haussmann *et al*. (2002) were used to survey the polymorphism between 100 genotypes and the list of primers surveyed is given in Table 2. The polymerase chain reaction (PCR) was performed in a 15 µl mixture containing 25 ng of genomic DNA, 50 ng each of forward and reverse primers (Sigma Aldrich, Bangalore, India), 0.25 mM dNTPs, 0.02 U *Taq* polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India), 10X assay buffer (10 mM Tris-HCl pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$) and 2.5 mM $MgCl_2$. Amplifications were performed on a thermal cycler (PTC-100TM, MJ Research Inc., USA). The standardized amplification profile was: initial denaturation temperature of 94°C for 15 min followed by 10 cycles of denaturation at 94°C for 10 sec; primer annealing at 61-52°C for 20 sec (the annealing temperature for each cycle is reduced by 1°C) and primer extension at 72°C for 30 sec followed by 35 cycles of denaturation at 94°C for 10 sec; primer annealing at 54°C for 20 sec and primer extension at 72°C for 30 sec. The last PCR cycle was followed by a final extension at 72°C for 20 min.

Table 2. List of microsatellite primers used in the study

Marker	Forward Primer	Reverse Primer
Xtxp43	AGT CAC AGC ACA CTG CTT GTC	AAT TTA CCT GGC GCT CTG C
Xtxp296	CAG AAA TAA CAT ATA ATG ATG GGG TGA A	ATG CTG TTA TGA TTT AGA GCC TGT AGA GTT
Xtxp295	AAA TCA TGC ATC CAT GTT CGT CTT C	CTC CCG CTA CAA GAG TAC ATT CAT AGC TTA
Xtxp88	CGT GAA TCA GCG AGT GTT GG	TGC GTA ATG TTC CTG CTC
Xtxp1	TTG GCT TTT GTG GAG CTG	ACC CAG CAG CAC TAC ACT AC
Xtxp38	ACA AAC CGC GAC GAA GTA AC	ACA AGG CAA AGC ACA AAG C
Xtxp340	AGA ACT GTG CAT GTA TTC GTC A	AGA AAC TCC AAT TAT CAT CCA TCA
Xtxp208	AAG GCC GTG AGG ATG	AAG CAG CCA AGA GCA G
Xtxp8	ATA TGG AAG GAA GAA GCC GG	AAC ACA ACA TGC ACG CAT G
Xtxp23	AAT CAA CAA GAG CGG GAA AG	TTG AGA TTC GCT CCA CTC C
Xtxp231	GGA AAT CCA GGA TAG GGT	AGG CAA AGG GTC ATC A
Xtxp149	AGC CTT GCA TGA TGT TCC	GCT ATG CTT GGT GTG GG
Xtxp227	TGA AAG TTT TGG CAT TGA	TGT AGG ATA GCC CAG GTT
Xtxp278	GGG TTT CAA CTC TAG CCT ACC GAA CTT CCT	ATG CCT CAT CAT GGT TCG TTT TGC TT
Xtxp59	GAA ATC CAC GAT AGG GTA AGG	GAC CCA GAA TAG AAG AGA GG
Xtxp357	CGC AGA AAT ACG ATT G	GCT ATC TGG AGT AAC TGT GT

Fifteen microlitres of the reaction mixtures were size fractionated through 3% agarose gel by electrophoresis and stained with ethidium bromide. The SSR gel images were scanned using the Gel Doc 2000 Bio-Rad system (Bio-Rad Laboratories, Hercules, California, USA). The bands were sized and then binary coded by 1 or 0 for their presence or absence in each genotype. The scores were used to create a data matrix to analyze genetic relationship using the NTSYS-pc program version 2.02 (Exeter Software, New York, USA) as described by Rohlf (1990). Cluster analysis was based on similarity matrices obtained with the Unweighed pairgroup method (UPGMA) (Sokal and Michener, 1958). To measure the informativeness of the markers, the Polymorphism Information Content for each SSR was calculated according to the formula: PIC = $1-\sum pi^2$, where pi is the frequency of the i^{th} allele.

Results and Discussion

Of the 16 SSR primers surveyed, 10 primers were polymorphic and produced scorable, unambiguous markers. A total of 47 scorable alleles were generated by these 10 primers of which 44 were polymorphic (93.6%). The number of alleles produced by different primers ranged from three to seven with an average of 4.7 alleles per primer. The polymorphic loci clearly discriminated all the genotypes. A high level of polymorphism (93.6%) was observed among the 100 sorghum genotypes. The mean number of alleles per locus was 4.7, with allelic sizes in close agreement with reports in the literature (Brown *et al.*, 1996; Taramino *et al.*, 1997; Bhattramakki *et al.*, 2000; Agrama and Tuinstra, 2003).

Table 3. Cluster composition of 100 genotypes used in SSR analysis

Clusters	No. of accessions	Accessions
I	26	B35, TENKASI1, CO22, CO21, CO24, AS4289, VS1560, CO1, K3, MS73, AS5078, AS8444, VS1564, AS8038, AS5773, M. Local, U. Chloam, AS1038, MS7728, Local 3, AS6329, LS120, AS1041, MS7837, MS7843, AS2699
II	11	AS5058, AS9916, AS5779, MS5826, AS3284, CO25, AS2059, AS5052, AS3998, AS3283, MS7818
III	5	AS7742, AS8021, AS8262, AS6184, AS7759
IV	12	AS4294, AS6002, AS900, AS8735, MS7826, AS3289, AS9098, AS9783, AS4959, MS7819, MS5133, IS5379
V	2	AS9912, MS7806
VI	5	AS5055, AS321, AS557, AS4599, MS7804
VII	3	AS4107, AS4153, ACM6
VIII	5	AS668, AS4641, AS4242, AS4647, AS4654
IX	4	AS3776, AS4226, MS7861, MS7703
X	18	CO26, M35-1, AS1, MS7725, MS7862, AS817, AS4243, AS7738, AS2752
XI	9	AS8007, AS5573, MS7816, AS4265, MS7863, AS3285, AS391, AS5787, AS4567

Similarity index values derived from the polymorphic data gave the extent of genetic relatedness among accessions (data not shown).The similarity coefficients based on 10 polymormphic SSR markers ranged from 0.205 to 0.864. Among the 100 accessions, B35 and Tenkasi 1 showed the highest similarity index (0.864), while the genotypes AS 4289 and CO 26, showed the least similarity index (0.205). The genotypes AS 4289 and CO 26 varied considerably in drought and yield component traits and these genotypes could be useful for hybridization, since hybrid vigor has a positive relation with genetic distance (Xiao *et al*., 1996). Hybridization between these two lines will provide progenies with greater variation at molecular level. The similarity coefficients were used as input data for cluster analysis using NTSYSpc2 program and the resulting dendrogram is shown in Figure 1.

The dendrogram grouped the sorghum accessions into 11 major clusters (Table 3). Cluster I comprises maximum number of genotypes (26) followed by cluster X (18), cluster IV (12), cluster II (11) and cluster XI (9). Cluster VIII, cluster III, and cluster VI had five genotypes each. Cluster IX, cluster VII and cluster V had four, three and two genotypes, respectively. Cluster analysis categorized the drought-resistant and drought-susceptible genotypes into separate clusters. These genotypes which were grouped into different clusters, were compared for their visual stay-green ratings, made during the field level studies to know the level of expression of stay-green genes under molecular level.

Most of the genotypes in the cluster I and IV had the stay-green score ranging between 1.80 to 2.5. Cluster IV genotypes are likely to have the same level of *per se* non-senescence as do the cluster I genotypes, despite being grouped into different clusters. These sorghum accessions may be suitable for growing under drought -prone tracts and rainfed areas. Most of the genotypes in the Clusters II, III, VII, IX and XI appeared to have moderate level of stay-green with a score ranging between 2.6 to 3.5. Cluster V, VI, VIII and X contained the most senescent genotypes which had the stay-green score ranging between 3.6 to 5. The current study included a wide range of genotypes representing more than one location but with same geographic origin. Hence, the clustering pattern of the accessions showed that though the genotypes represent a single geographical origin, there was greater molecular diversity among them. This is in agreement with the findings of Jeya Prakash (2006).

The stay-green specific primers used in this study "sorted the genotypes based on their level of drought tolerance" discriminating the genetic diversity among sorghum accessions. Hence, the genotypes which were sorted as stay-green in this study can be used as the parents in the hybridization programme for drought tolerance.

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Genotypic Variation for Quantitative and Qualitative Traits in Asiatic and European Carrot (*Daucus carota* L. var. *sativa*)

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Forty eight diverse genotypes of Asiatic and European carrots were evaluated for 17 quantitative and qualitative traits of horticultural importance. The analysis of variance was highly significant for all the traits indicating the presence of wide variability in the genotypes. High heritability estimates were recorded for plant weight, root weight, total yield, marketable yield and juice content. High estimates of genetic advance were recorded for total yield and marketable yield. The high heritability, associated with high genetic advance as percentage of mean for total yield, marketable yield, plant weight, root weight and beta carotene content, were the most reliable selection parameters. PC-50, PC-5, PC-81, IPC-122 and CT-2 were superior genotypes for yield and its components, and these can be used in further breeding programmes.

Key Words: Agronomic and biochemical traits, Carrot, *Daucus carota* L. var. *sativa*, Genetic variability

Introduction

Carrot (*Daucus carota* L.) 2n=2x=18 belongs to family Apiaceae and is native of Afghanistan (Banga, 1976). It is the most widely grown vegetable among root crops in India wherein it occupies an area of 24,000 ha with a production of 350,000 MT and an average yield of 145.83 q/ha (Anonymous, 2003). Carrots are an important source of pro-vitamin A, fiber and other dietary nutrients (Simon, 1990). According to the WHO, vitamin A deficiency partially or totally blinds nearly 350,000 children in more than 75 countries every year. Parameters of genotypic and phenotypic coefficients of variation (GCV and PCV) are useful in detecting the amount of variability present in the available germplasm. Heritability and genetic advance help in determining the influence of environment in expression of the characters and the extent to which improvement is possible after selection (Robinson *et al*., 1949). Thus, the present study aims to evaluate the available genotypes and to conduct the variability studies for different morphological and quality components of the roots in terms of yield attributes and qualitative traits like total soluble solids (TSS), dry matter, carotene content and juice yield of some Asiatic and European genotypes/cultivars selected from Punjab Agricultural University (PAU), Ludhiana; Haryana Agricultural University (HAU), Hisar; Indian Institute of Vegetable Research (IIVR), Varanasi; Sher-e-Kashmir University of Agricultural Sciences and Technology (SKUAST), Kashmir and Indian Agricultural Research Institute (IARI), New Delhi.

Material and Methods

The experimental material included 48 entries belonging to yellow, red, black and orange colour root types. The details of genetic stocks are given in Table 1. All the genotypes were sown on ridges in a double row of 3 m length in each replication with 45 cm spacing between rows and 7.5 cm between plants, respectively. All the recommended package of practices was followed during the course of investigation for raising a good crop. The observations were recorded for yield attributing traits like top height, plant weight, root length, root weight, root girth, core girth, flesh thickness, root to top ratio, total yield and marketable yield as well as for qualitative traits like total soluble solids (TSS %), exterior root colour, core colour, forking, dry matter, carotene content and juice yield. Ten roots from each replication were selected randomly, pooled and composite samples were analyzed. Total soluble solids (TSS %) were determined using a hand refractometer. The colour of carrot roots was determined by the level of total carotenoids, accumulation of other specific pigments as well as the distribution of pigments between phloem and xylem was with the colour chart of Royal Horticultural Society, U.K. as the reference chart. Carotenoids were extracted in acetone and portioned in petroleum ether and quantified by

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Table 1. Diverse lines of carrot and their sources under study

Genotypes	Source
Amity's Carrot	SKUAST, Kashmir
CCA-05-01	IIVR, Varanasi
CT-2	IIVR, Varanasi
Early Nantes	Sutton & Sons
HC-1	HAU, Hisar
HC-100	HAU, Hisar
HC-199-1	HAU, Hisar
HCB-22-2	HAU, Hisar
HCO-4-2	HAU, Hisar
HCP-2	HAU, Hisar
HCY-183-1	HAU, Hisar
Hybrid -501	SKUAST, Kashmir
IPC-106	IARI, New Delhi
IPC-109	IARI, New Delhi
IPC-118	IARI, New Delhi
IPC-122	IARI, New Delhi
IPC-25	IARI, New Delhi
IPC-34	IARI, New Delhi
IPC-37	IARI, New Delhi
IPC-4	IARI, New Delhi
IPC-40	IARI, New Delhi
IPC-7	IARI, New Delhi
JKC	SKUAST, Kashmir
KTCTH-7	IARI, Katrain
KTCTH-8	IARI, Katrain
Nantes	IARI, New Delhi
PC-101	PAU, Ludhiana
PC-15	PAU, Ludhiana
PC-16	PAU, Ludhiana
PC-34	PAU, Ludhiana
PC-35-A	PAU, Ludhiana
PC-41	PAU, Ludhiana
PC-42	PAU, Ludhiana
PC-43	PAU, Ludhiana
PC-44	PAU, Ludhiana
PC-5	PAU, Ludhiana
PC-50	PAU, Ludhiana
PC-61	PAU, Ludhiana
PC-76	PAU, Ludhiana
PC-79	PAU, Ludhiana
PC-81	PAU, Ludhiana
PC-82	PAU, Ludhiana
PC-83	PAU, Ludhiana
PC-84	PAU, Ludhiana
PC-87	PAU, Ludhiana
PC-94	PAU, Ludhiana
PC-96	PAU, Ludhiana
PC-99	PAU, Ludhiana

measuring the absorbance at 452 nm in a UV-visible double beam spectrophotometer as per the standard procedure (Ranganna, 1986). The specific absorbance values (specific extinction coefficient) tabulated by Davies (1976), were used for the calculation of carotene. Total carotene was expressed as mg/100 g fresh weight of carrot. Analysis of variance was calculated as suggested by Panse and Sukhatme (1985). The phenotypic and genotypic coefficients of variation (PCV and GCV) were estimated (Burton, 1953). Heritability in the broad sense suggested by Allard (1960) and genetic advance (in terms of percentage of mean) were computed according to Johnson *et al*. (1955).

Results and Discussion

Range, mean and analysis of variance for different quantitative and qualitative traits are presented in Table 2. The mean sums of squares were highly significant for all traits, indicating the presence of wide variability in the genotypes studied. Top height ranged from 50.48 cm (Nantes) to 71.21 cm (CT-2), with a mean value of 63.44 cm. There was considerable variability with respect to plant height which ranged from 181.67 g (PC-94) to 390.00 g (PC-15). Maximum root length and root weight were reported in PC-5 (24.92 cm) and PC-50 (182.00 g) and minimum were recorded in IPC-25 (19.57 cm) and HC-199-1 (101.33g), respectively. Maximum root girth (3.21 cm) was recorded in PC-42 and minimum (2.58 cm) in HC-100, whereas maximum (1.32 cm) and minimum (0.77 cm) core girth were recorded in HC-1 and PC-44, respectively.

Maximum flesh thickness (2.34 cm) was recorded in PC-42 and minimum (1.52 cm) in HC-100, whereas maximum root to top ratio (1.67) and minimum (0.66) were recorded in PC-35-A and KTCTH-8, respectively. The total yield exhibited by the genotypes, under study, varied from 3.15 kg/plot (IPC-37) to 7.92 kg/plot (PC-50) with average yield being 4.59 kg/plot. Marketable yield varied from 2.60-6.85 kg/plot with Amity's carrot registering the lowest and PC-50 the highest. PC-83 exhibited maximum TSS (8.78%) whereas a minimum was exhibited by IPC-34 (6.25%). Maximum dry matter (8.78%) was recorded in PC-83 and minimum (6.77%) in PC-94. There was a wide range (2.31- 6.96 mg/100g) for average carotene content with the minimum and maximum being recorded in genotypes PC- 61 and Hybrid-501, respectively. Juice yield varied between 415.83-581.67 ml/kg of roots with maximum being in PC-5 and minimum in Early Nantes, respectively.

Table 2. Range, mean and analysis of variance for different characters in carrot as per pooled data analysis

Character	Range	Min.	Max.	Mean	CD at 5%
Top height (cm)	50.48 -71.21	Nantes	CT-2	63.44	5.34
Plant weight (g)	181.67-390.00	PC-94	PC-15	260.34	18.78
Root length (cm)	19.57 -24.92	IPC-25	PC-5	21.89	1.26
Root weight (g)	101.33 -182.00	HC-199-1	PC-50	132.96	17.08
Root girth (cm)	2.58– 3.21	HC-100	PC-42	2.91	0.42
Core girth (cm)	0.77-1.32	PC-44	HC-1	0.98	0.26
Flesh thickness (cm)	1.52-2.34	HC-100	PC-42	1.87	0.41
Root to top ratio	0.66- 1.67	KTCTH-8	PC-35-A	1.49	0.26
Total yield (kg/plot)	3.15-7.92	IPC-37	PC-50	4.59	0.65
Marketable yield (kg/ plot)	2.60- 6.85	Amity's carrot	PC-50	3.64	0.85
Total soluble solids (%)	6.25- 8.78	IPC-34	PC-83	7.47	5.67
Dry matter (%)	6.77- 8.78	PC-94	PC-83	7.72	4.01
Carotene content (mg/100g)	2.31-6.96	PC-61	Hybrid-501	3.07	0.53
Juice yield (ml/kg)	455.83 -581.67	Early Nantes	PC-5	507.28	12.13

The pooled data is also represented in the form of a dendrogram (Fig. 1). Software package NTSYS PC version 2.02e (Rohlf, 1998) was used for estimation of genetic similarities among the lines using SIMQUAL mode of NTSYS. The similarity matrix value based on coefficient of similarity (Jaccard, 1909) was used to generate dendrogram. Clustering was done by UPGMA using SHAN module of NTSYS PC version 2.02e.

Knowledge of genetic diversity among population and its quantitative assessment usually helps a plant breeder in choosing desirable parents for a breeding programme. Genotypic diversity which has been generally considered as a criterion for the measure of genetic diversity in crop plants, very often fails to convey information about the genetic divergence.

In general, the phenotypic variance and phenotypic coefficient of variation were higher than the respective genotypic variance and genotypic coefficient of variation for all the traits (Table 3) indicating considerable influence of environment on their expression. Marketable yield had highest coefficient of variation at the genotypic and phenotypic level followed by total yield. Singh *et al.* (2004) also studied the coefficient of variation at the genotypic and phenotypic level, which was highest for average fresh weight of roots and root weight with leaves. Sizable coefficient of variation at genotypic and phenotypic (3-17%) levels was observed for carotene content, TSS, core girth and root length indicating high level of variability in these characters and ample scope for their effective improvement. Genotypic coefficient of variation would be more useful for assessing the variability.

Table 3. Coefficient of variation, heritability and genetic advance as % of mean for different qualitative and quantitative traits

Characters	GCV	PCV	Heritability (broad sense)	Genetic advance as % of mean
Top height (cm)	7.04	8.94	62.00	11.43
Plant weight(g)	22.61	23.05	96.30	45.71
Root length (cm)	3.83	10.59	13.10	2.83
Root weight (g)	21.72	23.19	87.70	41.91
Root girth (cm)	1.09	9.40	1.30	0.34
Core girth (cm)	4.40	23.38	3.50	2.04
Flesh thickness (cm)	4.63	12.48	13.80	0.53
Root to top ratio	2.11	14.69	2.10	4.70
Total yield (kg/plot)	33.06	34.35	92.60	65.58
Marketable yield (kg/ plot)	37.21	39.91	86.90	71.43
Total soluble solids (%)	8.5	10.22	69.20	14.59
Dry matter (%)	5.25	6.61	63.10	8.55
Carotene content (mg/100g)	13.65	17.21	62.90	22.47
Juice yield (ml/kg)	3.93	4.20	87.70	7.58

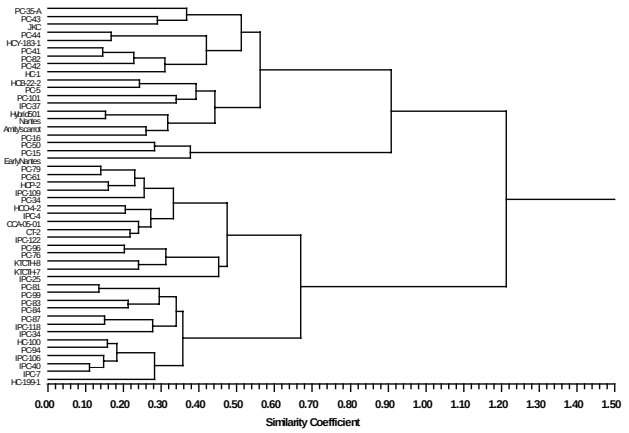

Fig. 1. Dendrogram on the basis of morphological studies using Jaccard's similarity coefficient

Burton (1953) has suggested that genotypic coefficient of variation together with heritability estimate would give the best option expected for selection. Heritability estimates were high in plant weight (96.30), total yield (92.60), root weight (87.70), juice yield (87.70) and marketable yield (86.90). High heritability for the characters controlled by polygene might be useful to plant breeder for making effective selection. Selection is always favored when a major proportion of a large amount of phenotypic variability is due to heritable variation. Heritability is a measure of genetic relationship between parent and progeny and has been widely used in determining the degree to which a character may be transmitted from parents to offspring. Knowledge of the degree of heritability for the character permits a rational choice of breeding methods to be followed for its improvement and helps to estimate the genetic gains from selection. Johnson *et al.* (1955) reported that the heritability estimates along with genetic advance are more useful than the heritability alone in predicting the resultant effect for selecting the best genotype as it suggests the presence of additive gene effect (Panse, 1957). High estimates of genetic advance as percentage of mean were recorded for marketable yield (71.43) and total yield (65.58). Similar results were also reported by Singh *et al*. (2004) and Tewatia *et al.* (1990). The high heritability was associated with high genetic advance as percent of mean for marketable yield, total yield, plant weight and root weight. The parallelism between the magnitude of heritability and degree of genetic gain has been due to the additive genes playing a predominant role. Presence of high and moderate heritability with low genetic gain suggested that high heritability did not necessarily lead to increased genetic gain unless sufficient genetic variability existed in the genotypes.

Occurrence of sufficient genetic variability for yield *per se* and its contributing traits, in the present set of genotypes can be exploited for carrot improvement. Marketable yield, total yield, plant weight, root weight and juice yield were the most reliable selection parameters. On the basis of above finding, PC-5, PC-15, PC-50, PC-42, PC-44, PC-35-A and Hybrid-501 were recorded to be superior genotypes for yield *per se* and its components traits, which can be used in future breeding programmes.

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Genetic Parameter and Association Analysis for Resistance to *Sclerotium rolfsii* Sacc. in Groundnut (*Arachis hypogaea* L.)

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Evaluation of 165 groundnut genotypes and two check parents (TAG-24 and R-9227) was carried out under artificial inoculation conditions for stem rot, *Sclerotium rolfsii.* indicated significant difference among genotypes, season and genotypes x seasons interaction for disease, yield and yield-related parameters. The genotypic and phenotypic coefficient of variation was high (>20%) for secondary branches, disease incidence at 30, 60, 90 days after sowing and pod yield/plant, moderate (10-20%) for plant height, leaf length and leaf width and low (<10%) for oil content and test weight. Heritability was high (>60%) for most of the characters. Genetic advance was high (>20%) for plant height, secondary branches, pod yield per plant and disease at 30, 60 and 90 days after sowing. Diesease incidence at harvest was negatively associated with plant population, shelling percentage, test weight, primary branches, secondary branches, leaf length and leaf width. Among the parents, R-9227 showed resistance to stem and pod rot, where as TAG-24 showed susceptibility to stem and podrot incidence.

Key Words: Association analysis, Groundnut, Heritability, Resistance, *Sclerotium rolfsii,* Variability

Introduction

Groundnut (*Arachis hypogaea* L.) is one of the most important oil seed crops and grain legumes grown worldwide. The groundnut seed has dual advantage of being important as a source of edible oil as well as protein. The exploitation of genetic resources from wild species is extremely difficult because of ploidy differences between cultivated tetraploid and diploid wild species coupled with compatibility barrier except with *Arachis* section. Obviously, poor soil fertility, and stem and pod rot disease caused by *Sclerotium rolfsii* Sacc.is one of the significant factors contributing to yield loss.

Only limited screening of germplasm for resistance has been attempted. There are very few reports of clear varietal differences for resistance to stem and pod rots. Although, no genotype is known to be immune or even highly resistant to *S. rolfsii*, several genotypes and advanced breeding lines have shown field resistance (Smith *et al.*, 1989; Grichar and Smith, 1992; Shokes *et al.*, 1993).

As in the case of many other diseases, breeding for disease resistance is the best way of controlling the *S. rolfsii*. To initiate breeding programme for resistance to any disease, understanding basic mechanism of disease resistance and its inheritance area pre-requisite. It is desirable to have a variety resistant to the disease, combined with other desirable yield characters. The knowledge of mode of inheritance and variability of resistance/susceptibility is essential to have effective selection programme. Estimate of gene effects will help in predicting the effectiveness of selection. The relative variance will decide the breeding procedure to be followed through the information available on the quantitative characters. Less information is available about the inheritance of *S. rolfsii* resistance as well as its association with other morphological traits.

The information searched thus far indicated the existence of variation in the pathogen. Further, pathogenic variability adds difficulties in effective management of this economically important disease. The situation also demands for the adoption of suitable breeding strategy in development of resistant varieties. Nevertheless, systematic study regarding the variability in the pathogen and other details are limited. Hence, to study the variability in *S. rolfsii* causing stem rot of groundnut, the present investigation was undertaken encompassing the objective of knowing the genetic parameter and association analysis for resistance to *S. rolfsii* in groundnut.

Material and Methods

The experimental material comprised of TAG-24 × R-9227 cross. This cross was made by using susceptible (TAG-24) and resistant (R-9227) parents in Randomized Complete Block Design. Hybridizations were forwarded to get F_1 (F_2 seeds) and F_2 generation (F_3 seeds) by selfing. The F_2 generation was advanced to F_3 through

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single seed decent method. Individual F_3 families were propagated as bulk in F_4 and F_5 generations. From F_5 generations, seeds were selected and evaluated in F_6 generations and artificial inoculation conditions were created during summer 2009.

S. rolfsii was isolated from infected groundnut plant grown in vertisols and mass multiplication was carried out on sand – corn meal medium (95:5) in order to get maximum sclerotial production (Abeygunwardhana and Wood, 1975). Two hundred gram of sand-corn meal medium was taken in 500 ml conical flasks and mixed with 30% distilled water and it was sterilized. The pure culture of isolated *S. rolfsii* was inoculated under aseptic conditions and incubated at 27 ± 1^0C for 30 days. These flasks were shaken frequently to get uniform growth of mycelium. The mass culture thus obtained was used for further studies. Inoculum containing mycelium and sclerotia along with corn meal and sand was applied to the soil surface around the base of the plants @ 125 g/2.5 m row, at 30 days after sowing or at flower initiation. Tie hopped sorghum stubbles (3–4 cm pieces) were scattered along the rows to enhance the fungal growth on soil. After two weeks, the inoculation was repeated. During summer season, the field was irrigated at five days interval until pod formation, to promote stem rot development. The observations were recorded for parameters like initial plant count, plant height, number of primary branches, number of secondary branches, leaf length and width, pod yield/plant, test weight, shelling (%), oil content (%), disease incidence (%) at various stages of crop growth and at harvest. Statistical analysis was done on mean, range, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability and genetic advance as per cent over mean (GAM) by using GENERS SPAR statistical package.

Results and Discussion

Plant breeders are mainly concerned with identification of important factors limiting productivity and formulation of appropriate breeding strategies to develop suitable genotypes. The average yield of groundnut in India (926 kg/ha) remains well below the levels achieved in countries like USA (2995 kg/ha). Several reasons are attributed for low yield level. Lack of improved high yielding cultivars, low soil fertility, uneven rainfall distribution, continuous cropping without crop rotation, low plant population and incidence of pests and diseases are major limiting factors in most of the groundnut growing regions.

Among the biotic stresses, stem and pod rot disease is predominant, accounting for yield losses to the extent of 10 – 25% and up to 80% in severely infected fields. Only limited resistance screening of germplasm has been attempted as there are very few reports of clear varietal difference with respect to resistance to stem and pod rot. None of the lines tested were completely resistant to stem and pod rot. It is unlikely that high degree of resistance to a highly necrotrophic pathogen such as *S. rolfsii* will be available. It would be desirable to select or develop lines that possess moderate resistance to *S. rolfsii* and resistance to other economically important diseases of groundnut.

The mean performance of segregating progenies with respect to 11 quantitative characters in *kharif* and nine characters in *summer* were studied. Plant height is the important component character in groundnut. The mean value of plant height was higher in case of F_5 population. It is because of good rainy condition for crop growth in *kharif* (F_5) compared to *summer* (F_6) population.

Secondary branches, leaf length, leaf width and mean values were similar or nearer in both F_5 and F_6 population. Hence, there was no difference among the genotypes. It is, thus, not advisable to use this parameter as a selection criteria.

In case of F_6 population, the mean value of disease incidence was low at 30 days after sowing (DAS), high at 60 DAS and moderate at 90 DAS. The range of disease incidence at 30 DAS, 60 DAS and 90 DAS was widened for this trait in F_6 population, because of application of artificial inocula, creation of artificial humidity and temperature by covering thin plastic sheet over experimental plot which favoured the spread of disease. It could be seen in the present study that there has been a change in mean value that accompanied the change in range also. This was evident for disease at harvest in both F_5 and F_6 progeny. The increase in mean value with increased upper unit of range of a character could offer better scope for selection.

The mean of test weight, shelling percentage, oil content, and pod yield per plant of *kharif* population were higher than those of *summer* population. It is indicated that during *summer* yield loss is greater compared to that during *kharif* season because high temperature and lack of irrigation leads to more root and pod rot disease.

Another measure of variation *i.e.* range displayed shift in its limits due to the trend of mean value. The range value widened for traits like plant height, pod-yield, shelling percentage and test weight during both the seasons indicated the release of concealed variability. Thus it has led to opening up broad spectrum of variation in above mentioned traits. The release of hidden variability could be of great use in improving the related traits through modifying breeding approaches (Singh and Balyan, 1988; Sharma *et al.*, 1995; Singh and Sahu, 1981).

The range of disease incidence at 30 DAS, 60 DAS and 90 DAS widened for this trait in F_6 population due to application of artificial inocula, creation of high humidity and temperature by covering experimental plot with thin plastic sheet which favoured spread of disease.

It could be seen in the present study that there has been a change in the mean value which accompanied the change in range also. This was evident for disease at harvest in both F_5 and F_6 progeny. The increase in mean value increased upper unit of range of a character, which offered better scope for selection.

Range of some traits like oil content, leaf length, and leaf width did not show any change, there by, indicating the operation of strong linkage between the traits. Under such circumstances a stringent selection following inter mating of high extreme segregants in the population would bring about breakage of tight-linked genes (Mather and Jinks, 1971). Narrow range was observed for plant height, plant population, primary branches and secondary branches.

Total variability observed in a crop can be divided into genotypic (Vg) and can be interpreted in terms of GCV and PCV (Table 1, 2 and 3). Coefficient of variance for F_5 and F_6 indicated the presence of less amount of variation for characters other then shelling percentage, disease incidence at harvest, pod weight per plant (GCV 10-20%) and high variability (>20%) (Badwal *et al.*, 1967; Nadaf and Habib, 1987). A comparison of GCV and PCV in F_5 and F_6 indicate estimated PCV were generally higher than GCV for all the characters. This may be due to involvement of high environmental and genotype × environmental interaction effects in character expression (Kaushik *et al.*, 1996).

High magnitude GCV and PCV was observed in case of secondary branches in F_5 and pod yield/plant, disease incidence at 30 DAS, 60 DAS and 90 DAS was widened for this trait in F_6 population. Higher magnitude of PCV and GCV for these characters in both F_5 and F_6 populations indicate the presence of high degree of variability and better scope for improvement.

Table 1. Genetic components of variance for various parameters of *Kharif* 2008

Characters	*Kharif* 2008					
	Mean	Range	PCV	GCV	h^2	GAM
Plant population	22.31	14.00-26.50	13.60	5.53	16.60	4.03
Plant height	21.75	15.62-30.97	13.99	12.69	82.30	20.50
Primary branches	6.39	4.50-8.75	15.67	6.57	17.60	8.60
Secondary branches	1.45	0.5-5.0	52.88	52.62	99.00	87.58
Leaf length	4.06	3.05-5.45	13.39	7.03	27.60	9.11
Leaf width	1.73	1.27-2.45	17.22	7.36	18.30	15.60
Test weight	43.75	33.00-53.50	14.72	7.64	26.90	7.74
Shelling (%)	69.10	57.67-76.87	16.78	7.48	19.90	5.70
Oil content (%)	43.14	39.10-46.93	3.90	2.98	58.30	4.68
Disease incidence at harvest	11.07	0.00-40.00	68.94	17.22	62.00	8.85
Pod weight/ plant	11.17	7.29-19.45	38.80	35.80	85.20	65.75

PCV=Phenotypic coefficient of variation, GCV=Genotypic coefficient variation, h^2=Heritability, GAM=Genetic advance over mean

In F_5 population the leaf width, leaf length, test weight, shelling percentage has shown moderate PCV and low GCV for both characters was observed. Improvement of GCV in F_5 population can be brought about by hybridization or selection in advanced generations.

Oil content of F_5 and shelling percentage in F_6 generation showed low PCV and GCV indicates that this character showed genetically much variation. Disease incidence at harvest in F_5 population exhibited high PCV and moderate GCV was observed. It indicates greater phenotypic variation compared to genotypic variation. Higher magnitude of PCV for this character indicates the presence of high degree of variability and better scope for improvement, but in F_6 population, high magnitude of PCV and low magnitude of GCV. This indicated the presence of high degree of variability and better scope for improvement.

In F_5 and F_6 populations, pod weight per plant with higher magnitude of PCV and GCV was obsened. Higher PCV and GCV were observed in F_5 population compared to F_6 owing to disease incidence in the F_6 population compared to F_5 population and resulting less yield in F_6 population.

Table 2. Genetic components of variance for various parameters of *summer* 2009 season

Characters	TAG-24		R-9227		*Summer* 2009			
	Mean	Range	Mean	Range	PCV	GCV	h^2	GAM
Plant population	25.50	25.00-26.00	19.00	14.24	13.03	5.12	15.50	4.60
Pod weight per Plant	10.25	9.60-10.90	11.10	10.4-11.80	23.55	20.58	76.40	35.45
Shelling percentage	76.37	76.25-76.50	72.25	72.00-72.50	7.82	3.95	25.60	9.14
Test weight	43.50	43.00-44.00	43.50	43.00-44.00	12.09	4.86	16.20	8.19
Oil content	44.68	44.51-44.85	41.85	41.33-42.37	3.86	3.02	61.10	5.81
Disease incidence at 30 DAS	0.50	0.00-1.00	0.55	0.00-1.00	88.66	87.82	98.10	180.80
Disease incidence at 60 DAS	4.00	1.00-7.00	1.50	1.00-2.00	82.92	81.39	96.40	164.70
Disease incidence at 90 DAS	3.00	2.00-4.00	0.00	0.00-0.00	61.61	61.49	99.60	126.90
Disease incidence at harvest	19.88	11.76-28.00	6.26	0.00-12.50	22.38	6.36	81.00	3.71

PCV=Phenotypic coefficient of variation, GCV=Genotypic coefficient variation, h^2=Heritability, GAM=Genetic advance over mean

The genotypes differed significantly for most of the parameters and reaction to disease. This indicated usefulness of this material and their appropriateness in the study. Over seasons, significant seasonal variation and genotypic × season interaction was observed for majority of the traits due to impact of environment and genotype × environment interaction. Wynne and Isleib (1978) reported extensive prevalence of G × E interaction for productivity traits, while Knauff and Gorbert (1993), and Wynne and Isleib (1978) indicated for physical traits. In general, variance estimate were relatively high in individual season as compared to pooled data indicating the role of G × E interactions.

Heritability estimates were useful while making selection based on phenotype. Nevertheless, as pointed out by Johnson *et al.* (1955), it would be limited as it is prone to change with fluctuation in season, environment, material *etc.* So, the estimation of heritability, thus, has a role in determining the effectiveness of selection in both early and advanced segregating generations. These are considered in conjunction with predicted genetic advance as suggested by Panse and Sukhatme (1967).

The high heritability with high genetic advance as percent over mean (GAM) were observed for traits like plant height, secondary branches, pod yield per plant, disease incidence at 30 DAS, 60 DAS and 90 DAS in both F_5 and F_6 populations. This indicated that there was lower environmental influence on the expression of these characters and it was governed by additive gene action and hence one can practice selection. Similar findings were reported by Reddy *et al.* (1985) and Hazara and Basu (2000) in okra.

Further, selection can be more effective in F_6 population because it exhibited higher variability for yield components as compared to F_5 which displayed low to moderate variability for various characters as these were showing less segregation for different characters. The F_5 population showed higher magnitude of variability, heritability and GAM for yield.

Low heritability and low GAM was observed in plant population, primary branches, test weight and shelling percentage in both F_5 and F_6 populations.

High heritability and low GAM was observed with respect to disease incidence at harvest and also for oil content in both F_5 and F_6 populations. It indicates that, these were controlled to a greater extent by non-additive gene action; it could be attributed to low genetic variability. Low GAM reflects increased effect of environment on these traits and thus selection procedures involving progeny testing may be followed to improve them.

Genetic correlation among different characters of a plant often arises because of linkage or pleiotropy (Horland and Csinos, 1939). Hence, the study of character association through correlation will help in selecting the yield attribute. The association between two characters can be ascertained by phenotypic correlation which

Table 3. Genetic components of variance for various parameters over seasons

Characters	Pooled analysis					
	Mean	Range	PCV	GCV	h^2	GAM
Plant population	20.89	11.0-28.0	13.11	4.81	13.5	3.64
Pod weight/Plant	11.52	3.93-30.40	13.26	1.27	23.52	2.17
Shelling (%)	63.53	30.50-78.70	11.26	2.41	0.46	1.05
Test weight	42.62	26.50-60.0	12.07	2.48	0.42	1.05
Oil content	43.15	38.52-49.16	4.25	3.47	6.64	5.81
Disease at harvest	41.60	0.00-100	29.15	0.08	51.65	0.84

PCV Phenotypic coefficient of variation, GCV Genotypic coefficient of variation, h^2=Heritability, GAM=Genetic advance over mean

Table 4. Genotypic and phenotypic correlations of *kharif* 2008

Character	PP	PH	PB	SB	LL	LW	TW	SP	OC	DIAH	PWP
Plant population	1.000	-0.307*	0.348*	-0.073	-0.942*	-0.951*	-0.344*	0.184*	0.372*	0.483*	-0.332*
		-0.117**	0.016	-0.025	-0.164*	-0.131**	-0.061	0.071	0.080	0.206*	-0.152**
Plant height		1.000	-0.151**	0.057	0.354*	0.248*	0.155**	0.201*	-0.165*	-0.708*	-0.304*
			-0.049	0.051	0.200*	0.090	0.083	0.059	-0.129**	-0.124**	-0.239*
Primary branches			1.000	-0.078	-0.662*	-0.589*	-0.340*	-0.107	0.030	0.083	0.267*
				-0.036	-0.099	-0.091	0.058	-0.027	0.078	-0.031	0.086
Secondary branches				1.000	0.062	-0.166 *	0.017	-0.036	0.135**	-0.098	-0.114**
					0.032	-0.069	0.005	-0.024	0.107	-0.013	-0.107
Leaf length					1.000	0.566*	-0.098	0.048	-0.202*	-0.339*	-0.325*
						0.617*	0.030	0.004	-0.024	-0.090	-0.133**
Leaf width						1.000	0.324*	-0.196*	0.006	0.108	-0.187*
							0.037	-0.097	0.033	0.005	-0.006
Test weight							1.000	0.623*	0.109	-0.225*	0.051
								0.269*	0.033	0.039	0.058
Shelling (%)								1.000	0.114**	-0.533*	0.351*
									0.014	-0.046	0.224*
Oil content (%)									1.000	-0.576*	0.146**
										0.102	0.092
Disease incidence at harvest										1.000	-0.346*
											-0.330*
Pod weight/plant											1.000

** Significance at 5% and 1% Propability
PP= Plant population; PH= Plant height; PB= Primary branches; SB= Secondary branches; LL= Leaf length; LW= Leaf width; TW= Test weight; SP= Shelling percentage; OC= Oil content; DIAH= Disease incidence at harvest; PWP= Pod weight/plant

is determined by measurement of two characters in a number of individuals of the segregating population.

In the present study, phenotypic and genotypic correlations were studied for pod weight per plant and its component traits in F_5 and F_6 populations. Phenotypic correlation of plant population, primary branches, test weight, shelling percentage and oil content exhibited positive significant association with pod weight per plant. Similar results were reported for primary branches (Badwal *et al.,* 1967; Sharma and Varshney, 1990; Nagabhushanam and Prasad, 1992), for shelling percentage (Pushkaran and Nair, 1993; Sharma *et al.,* 1995) and 100-seed weight (Sarala and Gowda, 1998; Nagda *et al.,* 2001) and were positively correlated with yield (Tables 4 and 5).

In all the populations, pod weight per plant had negative correlation with plant population, plant height (except F_6) and disease incidence at harvest. This indicates the possibility of identifying and isolating genotype with lesser plant population and lower disease incidence.

The characters *viz*., plant population, plant height, primary branches, secondary branches, leaf length, leaf width, test weight, shelling percentage, oil content and disease incidence at harvest not only exhibited significant association with pod weight per plant, but also showed significant positive association among themselves.

This character can thus be considered while selecting plants for high yield in F_5 and F_6 populations.

In case of F_6 population, disease incidence at 30 DAS was negatively correlated with plant population and with no significant effect on oil content, shelling percentage and pod weight per plant. Similar results were reported by Grichar and Smith (1992). Disease incidence at 30, 60 and 90 DAS was negatively correlated with plant population. This indicates that the disease incidence leads to reduction in plant population and yield components (Smith *et al.,* 1989).

The 100-seed weight was positively correlated with shelling percentage. Similar results were observed by Ramanathan and Raman (1968). Test weight had significant positive correlation with shelling percentage and oil content. Similar results were reported for shelling percentage (Ramanathan and Raman, 1968; Sangha, 1973) and disease incidence at harvest was positively correlated with plant population and negatively correlated with plant height, number of primary branches, oil content and pod yield per plant. Similar results were reported by Nagaraj (2003).

Table 5. Genotypic and phynotypic correlations of Summer 2009

Character	PP	PWP	SP	TW	OC	DI 30	DI 60	DI 90	DIAH
Plant population (PP)	1.000	-0.702** -0.288**	0.352** 0.060	-0.139 -0.021	-0.035 0.020	-0.563** -0.217**	-0.690** -0.249**	-0.613** -0.241**	-0.116 -0.200**
Pod weight/plant (PWP)		1.000	-0.174** -0.011	0.048 0.035	-0.160* -0.138	-0.029 -0.025	0.049 0.047	-0.032 -0.028	0.179** 0.040
Shelling percentage (SP)			1.000	-0.402** 0.069	-0.016 0.057	-0.167** -0.084	-0.041 -0.026	0.015 0.003	-0.058 0.098
Test weight (TW)				1.000	-0.295** -0.078	0.044 0.009	0.014 0.006	-0.029 -0.011	-0.497** 0.001
Oil content (OC)					1.000	-0.044 -0.042	-0.078 -0.047	0.113 0.082	0.133 0.012
Disease incidence at 30 days (D 30)						1.000	-0.178** -0.173**	-0.224** -0.222**	-0.009 -0.273**
Disease incidence at 60 days (D 60)							1.000	-0.300** -0.294**	-0.458** -0.400**
Disease incidence at 90 days (D90)								1.000	-0.100 -0.308**
Disease incidence at harvest (DIAH)									1.000

** Significance at 5% and 1% Propability

PP= Plant population; PWP= Pod weight per plant; SP= Shelling percentage; TW= Test weight; OC= Oil content; DI 30= Disease incidence at 30 days; DI 60= Disease incidence at 60 days; DI 90= Disease incidence at 90 days; DIAH= Disease incidence at harvest

Conclusion

It can be concluded that the studies clearly demonstrated genotypic difference in crop susceptibility to disease incidence and its parameters. As revealed by PCV, GCV, Heritability (h^2), GAM, this variation was highly heritable and thus existance of scope of selection. Among different characters, disease incidence at harvest and yield per plant exhibited substantial genotype x seasonal interaction as revealed by depressed genetic components of variance in pooled analysis. This indicates need for caution in selection for yield potential and disease incidence. In case of groundnut, *S. rolfsii* is a major disease which can cause up to 100% yield losses. There is a need for breeding for resistance to *S. rolfsii* since it is showing continuous negative association with all components of yield in groundnut.

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SHORT COMMUNICATION

Morpho-Physiological Evaluation of Chickpea (*Cicer arietinum* L.) Cultivars Under Restricted Soil Moisture Conditions

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A field experiment was conducted to evaluate the performance of 12 cultivar of chickpea released from IARI including six *desi* type namely, BGD 72, Pusa 256, Pusa 362, Pusa 372, Pusa 391 Pusa 1103 and six *kabuli* type namely, Pusa 1003, Pusa 1053, Pusa 1088, Pusa 1105, Pusa 1108, Pusa 2024 were evaluated under restricted soil moisture conditions. On the basis of seed yield performance, Pusa 1105, Pusa 2024, Pusa 1053 and BGD 72 were found superior as compared other cultivars tested. Membrane Stability Index (MSI) and Relative Water content (RWC) were recorded relatively higher in BGD 72, Pusa 1103. The above cultivar may be recommended for cultivation in North-West Plains Zone under restricted soil moisture conditions. BGD 72, Pusa 1103 and Pusa 2024 are also suitable to be used as parent in breeding for improving the drought tolerance in chickpea as these cultivars exhibited higher MSI and RWC values amongst all the cultivars.

Key Words: Chickpea, Drought tolerance, Membrane stability index, Relative water content, Seed yield

Chickpea is the most important food legume in India by virtue of its maximum contribution in area and production among the pulses. India grows chickpea on about 8.56 million hectares area producing about 7.35 million tonnes with a productivity of 858 kg/ha during 2009-2010 (http://www.aicrpchickpea.res.in). It is one of the most important food legume crop of the world and is grown extensively throughout most of the Indian sub-continent, North Africa, West Asia and in the Mediterranean regions. The chickpea is grown exclusively in the arid and semiarid zones of the world. In these areas, chickpea is continuously exposed to increasing drought and high temperatures during flowering and maturity stages due to insufficient and irregular rainfall (Toker *et al.*, 2007). About 90% of world's chickpea is grown under rainfed conditions where terminal drought is the major stress, accompanying with high temperature stress. The high temperature at post flowering phase results in forced maturity and reduced biomass production, several physiological, morphological and phenological traits may play a significant role in crop adaptation to drought stress during soil drying. Zaman-Allah (2011) found that tolerant genotypes possessed lower water uptake and a lower index of stomatal conductance at the vegetative stage as compared to sensitive ones, while tolerant genotypes extracted more water than sensitive genotypes after flowering. The other major constraints of low productivity are abiotic stresses salinity and nutrients. However moisture and high temperature stress are most important for limiting productivity of chickpea. The present study was conducted to evaluate the performance related to morpho-physiological traits of 12 cultivars with different parentage under restricted soil moisture conditions.

An experiment was conducted at research farm of Indian Agricultural Research Institute (IARI), New Delhi, 2009 and 2010 with 12 cultivar of chickpea including six *desi* types, namely, BGD 72, Pusa 256, Pusa 362, Pusa 372, Pusa 391 Pusa 1103 and six *kabuli* types, namely, Pusa 1003, Pusa 1053, Pusa 1088, Pusa 1105, Pusa 1108, Pusa 2024. The parentage of above mentioned cultivars are presented in Table 1. Soil was sandy loam with 7.8 pH and contained organic carbon 0.42% and available P 12.8 kg/ha. A basal dose of 20 kg N and 60 kg $P_2 O_5$ was applied at sowing. The crop was sown with three replications in Randomized Block Design. The crop was irrigated only at 70 days after sowing and then grown on restricted soil moisture conditions in the field. Observations were recorded for duration of different phenophases, namely, days to first flowering, days to 50% flowering, days to first pod formation, days to 50% pod formation and days to physiological

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Table 1. Pedigree of chickpea cultivars used in experiment

Type	Cultivar	Parentage
Desi	BGD 72	(Pusa 256 x E 100 YM) x Pusa 256
	Pusa 256	(JG 62 x 850 – 3/27) x (L – 550 x H 208)
	Pusa 362	(BG 203 x P 179) x BG 303
	Pusa 372	(P 1231 x P 1265)
	Pusa 391	(ICC 3935 x Pusa 256)
	Pusa 1103	(Pusa 256 x *Cicer reticulatum*) x Pusa 362
Kabuli	Pusa 1003	(ICCV 32 x Rabat)
	Pusa 1053	(ICCV 3 x FLIP 88 – 120)
	Pusa 1088	(Pusa 256 X ICCV 32) x ICCV 32
	Pusa 1105	(C 104 x BG 1003) x (ICC 88503 x BG 1048)
	Pusa 1108	(BG 315 x ILC 72) x (ICCV 13 x FLIP 85 – 11) x (ICCV 32 x Surutoto 77)
	Pusa 2024	(BG 261 x ICC 88503) x (GL 920 x BG 1003)

maturity. Data on yield and yield component traits namely, plant height (cm), number of primary branches, number of pods/plant, pod weight/plant (g), 100-seed weight (g), biological yield/plant (g), seed yield/plant (g) and harvest index (%) were also recorded under restricted soil moisture conditions. Membrane stability index (MSI) and relative water content (RWC) were estimated by following methods suggested by Deshmukh *et al.* (1991) and Barrs and Weatherlay, (1962), respectively.

Data recorded on different phenophases, MSI, RWC are presented in Table 2. Wide range of variations were recorded in all the phenophases of cultivars. Lower values of phenophases and higher values for MSI (%) and RWC (%) are the indicators for better performance under restricted water conditions. The *kabuli* type cultivars flowered earlier (56-66 days) as compared to *desi* types (62-72 days). Nearly similar trend was observed in days to 50% flowering also. In Pusa 1105, Pusa 2024, Pusa 1088 and Pusa 1003 achieved 50% pods relatively earlier (85-90 days) in comparison to other cultivars tested. No much significant differences were observed in physiological maturity amongst the two types of cultivars. However, *kabuli* type Pusa 1105 (145 days), and *desi* type Pusa 256 (146 days) were found as early maturing cultivars (Table 2). Nanda and Saini (1991) observed five days reduction in total flowering duration under restricted soil moisture. Earliness is a desirable trait for better adaptation to such environments, characterized by terminal drought and heat stress. It helps in drought escape whereby the crop completes its life cycle before the onset of terminal drought. It has been reported that the reduction of flower production due to water stress may reach up to 60% in chickpea (Fang *et al.*, 2010).

Membrane stability index values were significantly higher (76.17-78.47%) in BGD 72, Pusa 1103, Pusa 1108, and Pusa 256 (Table 2). Higher RWC (73.6-74.11%) were also recorded in BGD-72 and Pusa-1103. Gupta *et al.* (2000) observed that the genotypes, which were more tolerant to moisture stress, had lower membrane injury (less ion leakage) and low drought susceptibility index. Singh *et al.* (2004) observed that at pod formation stage all genotypes showed relatively higher membrane injury than at vegetative and flowering phases. This indicated natural senescence of leaves or more realization of stress during pod formation stage and also reported that BGD 72 and Pusa 1103 possessed significantly higher values of RWC, so these genotypes performed better under limited water environments. RWC was a measure of stress adaptation and accounts for osmotic adjustment, which is considered to be one of the important mechanism for adaptation to water limited environments in plants. BGD 72 registered high RWC than ICCV2 at the end point (Jain and Chattopadhyay, 2010).

The data on plant height, number of primary branches, number of pods/plant, pod weight/plant, 100-seed weight, biological yield/plant, seed yield/plant and harvest index (%) are presented in (Table 3). Plant height (cm) ranged from 63.87 to 85.00 in all the cultivars tested. Pusa 1105, Pusa 2024, Pusa 1053, and BGD 72 gave seed yield equal to or more than 24 g/plant; similarly higher number of pods/plant (84-96), pod weight/plant (32-34 g), 100-seed weight/plant (27-28 g) and biological yield/plant (55-66 g) were recorded (Table 3). High harvest index values were observed in all the cultivars mentioned above except BGD 72. It is evident that pods/plant, 100-seed weight and biological yield have contributed towards higher seed yield in the cultivars grown in restricted water conditions. Mwanamwenge *et al.* (1999) observed that maximizing the number of flowers, pods and seeds are the most important traits for maintaining stable and high seed yield under water deficit condition in Faba bean. The development of water deficit during the reproductive stage plays an important role in determining the number of flowers and pods that produce seed yield. Pod abortion was known to be important in determining seed yield of chickpea when exposed to terminal drought, but flower production and abortion were, important factors reducing seed yield (Fang *et al.*, 2010). Krishnamurthy *et al.* (1999)

Table 2. Duration of different phenophases, membrane stability index (MSI) and relative water content (RWC) of chickpea cultivars under restricted moisture conditions in the field

Type	Cultivar	Days to first flowering	Days to 50% flowering	Days to first pod formation	Days to 50% pod formation	Days to physiological maturity	MSI (%)	RWC (%)
Desi	BGD 72	66	75	89	104	155	78.47	74.11
	Pusa 256	66	75	86	96	146	76.17	67.63
	Pusa 362	62	75	87	93	155	64.00	58.80
	Pusa 372	71	75	88	99	156	55.27	58.00
	Pusa 391	72	82	89	95	154	65.47	63.43
	Pusa 1103	72	80	87	95	154	78.30	73.63
Kabuli	Pusa 1003	58	74	82	90	154	64.80	58.53
	Pusa 1053	58	76	88	104	155	61.80	62.30
	Pusa 1088	66	75	84	90	155	68.27	57.13
	Pusa 1105	56	67	76	85	145	54.76	56.97
	Pusa 1108	57	69	86	95	154	76.17	64.17
	Pusa 2024	56	70	84	90	153	68.90	61.63
CD 0.05)		2.16	2.67	1.56	4.22	2.27	2.02	1.61

Table 3. Yield and yield component traits of chickpea cultivars under restricted moisture conditions in the field

Type	Cultivar	Plant height (cm)	No. of primary branches	No. of pods/ plant	Pod weight/ plant(g)	100-seed weight (g)	Biological yield/ plant (g)	Seed yield/ plant (g)	Harvest index (%)
Desi	BGD 72	76.06	6	84.37	32.77	27.50	60.27	24.93	37.03
	Pusa 256	63.87	6	73.10	15.83	17.43	35.57	12.80	34.93
	Pusa 362	79.33	4	61.50	21.67	22.89	37.23	16.47	46.00
	Pusa 372	72.17	4	65.09	16.57	21.73	32.27	12.60	41.97
	Pusa 391	74.13	5	59.66	18.73	23.40	35.63	15.77	45.43
	Pusa 1103	82.53	6	52.60	19.77	22.67	32.47	15.03	43.43
Kabuli	Pusa 1003	71.43	4	33.40	10.80	21.93	22.69	8.72	37.63
	Pusa 1053	83.81	6	91.30	32.67	28.53	55.28	24.73	45.33
	Pusa 1088	85.07	4	57.47	29.33	25.63	47.77	22.17	42.67
	Pusa 1105	83.78	6	84.57	34.00	27.70	65.90	26.93	43.13
	Pusa 1108	76.21	5	67.03	28.57	25.87	42.30	17.67	38.93
	Pusa 2024	71.73	6	96.90	32.60	27.43	65.97	25.60	45.30
CD 0.05)		1.93	1.37	2.18	1.23	1.38	1.59	1.30	1.09

noticed that highest contribution of harvest index to seed yield has been demonstrated in chickpea only when the terminal drought conditions were severe. On the basis of seed yield data recorded, Pusa 1105, Pusa 2024, Pusa 1053, and BGD 72 were found promising in restricted soil moisture conditions. In general, MSI (%) and RWC (%) were also recorded relatively higher in BGD 72, Pusa 1103 and Pusa 2024. It is recommended that above cultivars may be grown in water restricted conditions in Northern Plain Zones particularly Delhi like conditions. Cultivars BGD 72, Pusa 1103 and Pusa 2024 may be used as parents in breeding programme for improving drought tolerance in chickpea. It is also observed that such morpho-physiological traits may be used as selection criteria for end season drought and terminal heat tolerance in chickpea breeding programme.

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SHORT COMMUNICATION

Identification of an Upright Peduncle and Podding Genotype in Chickpea Germplasm Conserved in the National Genebank

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A total of 18,873 accessions of chickpea germplasm were characterized in 2011-12, which were conserved in National Genebank at National Buearu of Plant Genetic Resources. IC486088 was found to bear upright peduncle and podding behaviour. This trait has special significance in breeding varieties amenable to mechanical harvesting to save labour requirement used for manual harvesting.

Key Words: Characterization, Chickpea, Upright podding

India is the largest chickpea producing country with a share of about 64% of global chickpea production (FAOSTAT, 2012). Chickpea is the most important pulse crop in India in terms of both area and production. During the last 10 years, although the productivity of chickpea has increased by 1.74%, but the gross chickpea production has gone up by 6.32% and the area by 4.43%. Consecutively for the third year in a row, there has been increase in production, with a record production of 8.25 Mt achieved during 2010-11 (AICRP Chickpea, 2011-12).

Cultivated chickpea (*Cicer arientinum* L.) has two distinct forms, *i.e. Desi* (small seeded, angular shaped and coloured seeds with high percentage of fibre) and *Kabuli* types (large seeded, owl shaped, beige coloured seeds with a low percentage of fibre). The average yield of chickpea is still low in India and further genetic improvement is constrained by the non-availability of appropriate germplasm. Further, there is a scope for widening the genetic base of cultivated varieties through pre-breeding. Plant genetic resources are the reservoir of useful gene/allele sources and provide basic raw materials for further genetic improvement. National Bureau of Plant Genetic Resources (NBPGR) is the nodal organization in the country and is actively involved in the plant genetic resources management since long through the germplasm exploration and collection, characterization, evaluation and conservation in the National Genebank.

Chickpea, being an important pulse crop, has received due attention in the introduction and conservation of genetic resources in India. So far, approx. 56,925 accessions including international nurseries/trials have been introduced from 56 countries (NBPGR, 2011-12). The International Centre for Agricultural Research in the Dry Areas (ICARDA), Syria, has been an important source of introduction from where about 15,880 accessions of chickpea were introduced. Currently, 14,061 base collections are conserved in the National Genebank under long-term storage at NBPGR, New Delhi, of which 11,438 are of indigenous and 2,623 are exotic origin conserved in the long-term storage (LTS) of the National Genebank.

The Bureau undertook a large scale characterization and evaluation programme of the entire chickpea germplasm (LTS) and some collections maintained under medium-term storage (MTS) at AICRP - Chickpea centre, Mahatma Phule Krishi Vidyapeeth (MPKV) Rahuri, Maharashtra, in a collaborative mode to enhance their proper utilization, during *rabi* 2011-12. A total of 18,873 germplasm accessions were grown under Augmented Block Design with three improved varieties, *viz.*– Vijay, Digvijay and Vishal as checks.

The observations were recorded on 20 agro-morphological traits, *viz.*– early plant vigour, plant growth

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Fig. 1. (a) Unique upright peduncle and podding (b) Normal droopy peduncle and podding

habit, plant pigmentation, number of leaflets/leaf, leaflet size (cm), plant pubescence, days to 50% flowering, flower colour, plant height (cm), number of primary branches/ plant, biomass score, number of pods/plant, pod shape, number of seeds/pod, days to 80% maturity, seed colour, testa texture, 100-seed weight (g) and seed yield/plant (g) as per chickpea minimal descriptors. While recording these various morphological and agronomic traits in the entire chickpea germplasm, one accession IC486088, was found to bear upright peduncle and podding behavior (Fig. 1a), in contrast to the generally observed podding behavior, which is of droopy nature (Fig. 1b). This unique trait was further validated in the off-season Himalayan nursery at Chaudhary Sarwan Kumar, Himachal Pradesh Agricultural University, Research Station, Sangla, H.P., during summer 2012, where the upright podding behavior was found to be true to its type. Different agro-morphological traits of this accession as compared to one of the check varieties Vijay, is given in Table 1. Field view of chickpea germplasm grown at Mahatma Phule Krishi Vidyapeeth, Rahuri during *rab*i 2011-12, and its further validation during off-season nursery (in pots) at Chaudhary Sarwan Kumar, Himachal Pradesh Agricultural University, Research Station Sangla, H.P. is presented in Fig. 2.

The upright podding trait has a special significance for its possible utility in breeding verities amenable to mechanical harvesting of chickpea which is becoming increasingly important in view of huge labour requirement for manual harvesting of crop and the associated drudgery involved with it. This unique accession bearing special

Table 1. Performance of the upright podding chickpea genotype, IC 486088 for various agro-morphological traits in comparison with the best check Vijay

S. No.	Geno-type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
		PLT_ VGR	GRW_ HAB	PLT_ PIG	LFLT_ LF	LFLT_ SIZ	PUB	DAY_ FLW	FLW_ CLR	PRI_ BRN	BIOM	PLT_ HT	POD_ PLT	POD_ SHP	SED_ POD	DAY_ MAT	SED_ CLR	SED_ SHP	SED_ SURF	GRN_ YLD	SED_ WGT
1	IC 486088	3	3	3	4	2	5	55	6	3.6	2	62.0	42.0	3	1.6	104	17	1	7	18.6	15.0
2	Vijay (check)	3	4		5	2	99	42	5	4.2	2	33	46.3	1	1.3	93	3	1	7	19.6	19.2

1	PLT_VGR	Early plant vigour	8	FLW_CLR	Flower colour	15	DAY_MAT	Days to 80% maturity
2	GRW_HAB	Plant growth habit	9	PRI_BRN	Number of primary branches/plant	16	SED_CLR	Seed colour
3	PLT_PIG	Plant pigmentation	10	BIOM	Biomass	17	SED_SHP	Seed shape
4	LFLT_LF	Number of leaflets/leaf	11	PLT_HT	Plant height (cm)	18	SED_SURF	Seed surface (testa texture)
5	LFLT_SIZ	Leaflet size (cm)	12	POD_PLT	Number of pods/plant	19	GRN_YLD	Grain yield/plant (g)
6	PUB	Plant pubescence	13	POD_SHP	Pod shape	20	SED_WGT	100-seed weight (g)
7	DAY_FLW	Days to 50% flowering	14	SED_POD	Number of seeds/pod			

Fig. 2. (a) Field view of chickpea germplasm grown at MPKV, Rahuri, Maharashtra,(b) chickpea accession, IC486088 showing upright podding behaviour in the field and (c) validation of upright podding trait during summer 2012 at CSKHPKV, RS, Sangla Himachal Pradesh

trait of interest can be further investigated to understand its inheritance pattern.

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SHORT COMMUNICATION

A Note on Fungi Intercepted on *Camptotheca acuminata* Decne Seeds from China

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Four accessions of *Camptotheca acuminata* Decne imported from China were processed for quarantine clearance. Quarantine processing revealed the presence of *Alternaria porri* (Ellis) Ciferri, *Curvularia lunata* (Wakker) Boedijn and *Periconia byssoides* Persoon and their significance is discussed along with the need for further research.

Key Words: *Alternaria porri*, *Camptotheca acuminata*, China, Fungi, Interception

Camptotheca acuminata Decne (Nyssaceae), commonly called as "Happy tree" or "Tree of joy", is a native plant of China. It has been used effectively in Chinese medicine for treating psoriasis, liver and stomach problems and common cold for many centuries. It was found to produce a potent antitumor alkaloid, called camptothecin (Wall *et al*., 1966). The bark of stem and root, and seeds can yield camptothecin (a pentacyclic quinoline alkaloid) in trace amounts, while the highest concentrations are found in tender young leaves (Lucas, 2009). The tree is now being considered as "*Endangered*" by the Government of China and export is restricted. Recently, seeds of four accessions of *C. acuminata* Decne were imported from China and processed for quarantine clearance. The paper deals with fungi intercepted along with their importance.

All samples were visually examined under stereo-binocular microscope and seeds suspected with infection from each sample were taken out and subjected to standard blotter test. Ten seeds were plated in each Petri dish with three replications/sample. After incubation for 7 days at 20 ± 1^0C under alternating cycles of 12 h light and darkness, plates were examined under stereo-binocular microscope for the presence of seed-borne pathogens, if any. Slides were made and examined on 8th day under compound microscope for specific identification of the pathogens. *Alternaria alternata* (Fr.) Keissl. and *Curvularia lunata* (Wakker) Boedijn were recorded in two accessions with infection percentage of 6.7 (EC687397) and 23 (EC687398). Long (2003) identified *A. alternata*, *Pestalotia guepinii* Desm (Steyaert), *Epicoccum nigrum* Link as leaf spot causing agents, while *Discula umbrinella* (Berk. & Br.) Morelet, *Drechslera* sp., and *Nectria* sp. (Fr.) Fr., and *Fusarium avenaceum* (Corda ex Fr.) Sacc. as root rot causing species in *C. acuminata*. Li *et al*. (2005) reported that plant diseases such as leaf spots and root rot are some of the major fungal diseases that can limit the yields of *C. acuminata* plants and decrease the production of camptothecin (CPT). It was also observed that these fungal diseases were less serious in some higher CPT yielding varieties. *C. lunata* is also responsible for leaf spot disease in many crops including pearl millet (Nitharwal *et al*., 1991).

Alternaria porri (Ellis) Ciferri was recorded in one accession of *C. acuminata* (EC687399). It is a seed-transmitted fungus, known to infect mainly onion, garlic and other *Allium* spp. The fungus incites purple blotch in onion, causing heavy yield loss ranging from 2.5 to 87.8% during *kharif* season (Srivastava *et al*., 1994). Seeds infected by this pathogen had a reduced germination.

Periconia byssoides Persoon was observed in one accession (EC687397) with 20% infection. This fungus is known to be parasitic causing leaf spots in castor (Karan, 1966) and stem blackening in hemp. *Cladosporium* sp Link: Fr. was recorded in all four accessions. Many species of *Cladosporium* are known to cause stem canker in hemp and other drug plants and among them the most common species is *C. herbarum* (Mc Partland *et al*., 2004). It also attacks leaves and seeds. *Acladium* sp., *Chaetomium* sp. Kunze, *Periconia byssoides* Persoon, *Cunninghamella* sp. Matruchot, and *Glomurularia* sp. were the other fungal species recorded on some accessions. Glomurularia leaf mold was recorded on *Gingko biloba*, a medicinal plant

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during 1998 in the Alabama plant diagnostic lab (http://www.ag.auburn.edu/hort/landscape/STGOnovember98.html).

There are reports on isolation of endophytic fungi from roots, branches, leaves and fruits of *C. acuminata*, some of which belong to *Alternaria*, *Colletotrichum*, *Diaporthe*, *Fusarium*, *Guignardia*, *Nigrospora*, *Paecilomyces*, *Penicillium*, *Pestalotiopsis*, *Phomopsis*, *Sporidesmium*, *Sordariomycete* and *Zythia* species (Ding *et al*., 2010). Preliminary screening of these fungal strains indicated that these endophytes can produce multi-kinds of metabolites including CPT (Yan *et al*., 2008). Kusari (2010) reported the occurrence of *Fusarium solani* (Mart.) Sacc., an endophyte from the bark of 'Happy tree' and identified its potential to produce CPT. Antimicrobial activities of these endophytes against pathogenic fungi such as *Rhizoctonia solani* Kuhn, *Gibberella fugikuroi* (Sawada) Wollenworth, *G. zeae* (Schwein.) Petch,, *Pyricularia grisea* (Cooke) Sacc., and *Fusarium oxysporum* f. sp. *vasinfectum* W.C. Snyder & H.N. Hansen, revealed the importance of these endophytes as novel bioactive compounds (Ding *et al*., 2010). Research achievements of the endophytic fungi isolated from anti cancer medicinal plants, and some of the isolates producing the same physiological active substances as product of the hosts are reviewed by several workers and emphasized the potential applications of the endophytic fungi (Zhou Chen *et al*., 2002). Further studies are required to ascertain the role of fungal species in the present study as pathogens of the host plant or endophytes that can help in producing CPT or exhibiting antimicrobial activities against pathogens.

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SHORT COMMUNICATION

Evaluation of Garden Pea Genotypes for Yield and Screening against Downy Mildew Incidence under Mid Hill Conditions of Jammu Region

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Downy mildew caused by [*Peronospora viciae* (Berk.) Casp.] is one of the most prevalent disease of garden pea and under mid hill conditions of Jammu region downy mildew causes severe economic losses to farmers. Twenty genotypes of garden pea were evaluated and screened against downy mildew disease at RARS, Rajouri, SKUAST-Jammu during *rabi* seasons of 2007-08 and 2008-09. Based on the results obtained from pooled data of two years, all the genotypes were grouped into five groups (Group I, II, III, IV and V). Among all the genotypes, P-89 was found to be the most suitable genotype with minimum downy mildew incidence (10.3%) and highest yield (217.5 q/ha) under mid hill conditions.

Key Words: Downy mildew, Garden pea, *Peronospora viciae*

Garden pea (*Pisum sativum* L.) is one of the foremost vegetable crops of the Jammu region. It is cultivated in 2300 hectare area with production of 20700 metric tonnes (Anonymous, 2009). Out of the total area under pea crop, majority area lies in mid hill zone comprising of Rajouri, Poonch, Bhaderwah, Kishtwar and Udhampur districts which is highly conducive for pea cultivation. However, in the recent past due to heavy yield losses in the prevalent pea cultivars and their susceptibility to the downy mildew disease [(*Peronospora viciae* (Berk.) Casp.] caused havoc amongst the vegetable growers of the region. Therefore, the present study was undertaken to find out new high yielding and downy mildew tolerant cultivars and in mitigating the problems of pea growers in this region.

The experiment was conducted during *rabi* season of 2007-08 and 2008-09 at research farm of SKUAST Regional Agricultural Research Station, Rajouri, Jammu. The soil of the experimental field was clay in texture with 196.5 kg/ha available N, 8.1 kg/ha P_2O_5, 124 kg/ha available K_2O and having a pH of 7.67. The experiment was laid out in randomized block design (RBD) having 20 treatments, each replicated thrice. The treatments comprised of twenty varieties of pea namely, CPS-05-01, CPS-05-02, CPS-05-03, CPS-05-04, CPS-05-05, AP-3, Palam priya, Arkel, VL-9, VL-8, E-8, AP-1, Early Gaint, Bonnevillae, Pusa Pragati, DMR-7, DDR-23, DDR-27, P-89 and DDR-55. Each variety is sown in a plot of size 3m X 1.5m at spacing of 40x20 cm. The fertilizer is applied at recommended dose of 50, 60 and 50 kg nitrogen, phosphorous and potassium per hectare. All the cultural operations were carried out uniformly in all the treatments as per package of practices recommended by SKUAST-Jammu (Anonymous, 2009). Observations on plant height, number of branches/plant, number of nodules /plant, number of pods/plant and pod weight/plant were recorded from five randomly selected plants in each plot whereas data on days to 50% flowering, average yield and downy mildew incidence were recorded on whole plot basis (Davidson *et al.*, 2011). The two year data was pooled and statistically analyzed following standard procedure (Snedecor and Cochran, 1967).

Perusal of pooled data for two years revealed superiority of P-89 among all the genotypes used in the study (Table 1). The overall superiority of P-89 was expressed in terms of high values of other yield contributing characters *viz.*, number of branches/plant (3.4), number of nodules/plant (63.1), number of pods/plant (31.3), and pod weight (198.3).The significant difference in terms of green pod yield was observed in P-89 (217.5 q/ha), CPS-05-03 (205.7 q/ha), Palam Priya (201.9 q/ha), AP-1 (193.2 q/ha) and was 31.4%, 24.3%, 22.0% and 16.7% respectively, higher than check cultivar Bonneville(165.5 q/ha). All the other

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Table 1. Incidence of downy mildew and agronomic traits of different genotypes of garden pea under mid hill conditions of Jammu region (pooled data for 2007-08 and 2008-09)

Genotype	Plant height (cm)	No. of branches/ plant	No. of nodules/ plant	No. of pods/ plant	Pod weight/ plant (g)	Days to 50% flowering	Yield/ha (q)	Downy mildew incidence (%)
CPS-05-01	151.6	4.2	52.8	36.5	135.3	80.3	166.1	10.5
CPS-05-02	101.4	3.4	30.4	29.5	115.1	84.7	192.0	17.7
CPS-05-04	92.3	2.8	61.2	30.5	102.1	83.2	175.9	17.0
CPS-05-05	108.3	3.0	42.8	27.9	138.3	80.2	182.4	13.5
CPS-05-03	112.2	4.3	63.5	48.5	153.9	81.7	205.7	15.7
AP-3	78.5	1.7	40.4	15.6	81.2	62.7	170.3	15.5
Palam Priya	86.3	3.0	77.1	40.7	130.1	81.8	201.9	17.2
Arkel	92.4	2.8	74.3	25.8	91.4	70.5	223.8	11.8
VL-9	105.6	2.9	49.7	19.5	87.2	73.8	182.6	14.5
E-6	70.7	2.1	42.9	25.4	71.9	60.2	187.3	11.9
VL-8	87.4	2.8	49.3	38.9	72.1	78.3	158.3	15.3
AP-1	93.2	3.6	65.0	45.8	141.0	78.8	193.2	14.5
Early Gaint	194.6	3.2	30.2	30.1	164.3	77.5	102.9	17.8
Bonneville	91.4	3.0	46.0	38.0	120.9	69.2	165.5	12.5
Pusa Pragati	84.1	2.4	48.9	19.9	89.2	74.8	131.4	10.5
DMR-7	144.5	3.2	60.5	26.3	120.5	80.5	102.6	13.0
DDR-23	95.6	3.7	67.7	32.0	122.3	67.7	118.3	15.2
DDR-27	81.5	2.4	39.0	17.3	83.0	74.7	114.9	13.2
P-89	77.3	3.4	63.1	31.3	198.3	75.0	217.5	10.3
DDR-55	69.8	2.0	41.7	13.4	45.3	87.3	70.3	13.8
CD (0.05)	14.72	0.66	7.47	6.07	12.98	8.37	10.4	1.46

genotypes performed poor in term of yield as compared to Bonneville. However, based on the parameter of earliness of flowering measured in terms of days to 50% flowering in genotype E-6 (60.2 days) followed by CPS-05-03 (62.7 days) and DDR-23 (67.7days) were recorded to be the mature significantly early when compared with check Bonneville (69.2days) whereas non significant differences were observed in P-89 (75 days) when compared with Bonneville for earliness.

Based on the two year pooled data presented in Table 1, it was revealed that significant variation was present among different genotypes regarding downy mildew incidence. All the genotypes exhibit varying degree of disease incidence ranging from 10.3% (P-89) to 17.8% (Early Giant). Based on the percentage disease incidence, all the genotypes were classified into five groups (Group I, II, III, IV, and V) (Table 2). Group I comprised of P-89, CPS-05-01, Pusa Pragati, Arkel, E-6 and Bonneville were found to be least susceptible to downy mildew (10.3-12.5%). Group II consisting four genotypes (CPS-05-05, DMR-7, DDR-27 and DDR-55) recorded downy mildew incidence between 13.0-13.8%. Similarly Group III (AP-3, VL-9, VL-8, AP-1 and DDR-23) and Group IV (CPS-05-03 and CPS-05-04) recorded disease incidence of 14.5-15.5% and 15.7-17.0%, respectively, where as Group V (CPS-05-02, Palam Priya and Early giant) exhibited maximum disease incidence (17.2-17.8%).

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