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Discerning genetic diversity among super early pigeonpea germplasm using microsatellite markers

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ABSTRACT

In the present study genetic diversity studies have been carried out using twenty four polymorphic SSR markers in a set of thirty five super early pigeonpea germplasm. The primers amplified a total of 69 alleles with 24 primers having highest PIC of 0.74. The SSR markers grouped the germplasm into four clusters based on jaccard's similarity coefficient. To test the goodness of fit of a clustering to a set of SSR data, cophenetic correlation coefficient or cophenetic value was estimated using the COPH and MXCOMP options in NTSYS-pc program. The cophenetic value of 0.94 obtained using the SSR data indicated a very good fit. The polymorphic SSR primers could clearly distinguish the determinate germplasm and non-determinate germplasm. PCA revealed that phenotypic variation explained by the SSRs with first four PCs accounted to 65.4% of total variation. Analysis of molecular variance of pigeonpea germplasm has shown that approximately 1.2% of genetic diversity was within super early germplasm, 71% among germplasm, and 27% among determinate and indeterminate types.

Key words: Genetic diversity, Microsatellites, Pigeonpea, Super early germplasm.

The unraveling of the genome sequence of pigeonpea along with deep transcriptome studies, has enriched us with vast genomic resources that assisted the molecular breeding efforts in pigeonpea (Dutta *et al.* 2011; Singh *et al.* 2012; Varshney *et al.* 2012). Morphological studies alone do not provide sufficient information to understand genetic diversity within the species as well as its relatedness to other species. Molecular analysis using SSRs can provide additional information on genetic diversity that would be useful for breeding programs through selection of diverse parents (Charcosset and Moreau 2004). Microsatellites offer several advantages compared to other molecular markers: they are highly reproducible, highly polymorphic, PCR-based and readily portable within a species (Edwards *et al.* 1996). Only limited pools of pigeonpea germplasm have been characterized earlier through RFLP (Sivaramakrishnan *et al.,* 2002), RAPD (Ratnaparkhe *et al.,* 1995), SSRs (Burns *et al.,* 2001; Odeny *et al.,* 2007), AFLP (Panguluri *et al.,* 2006) DArT (Yang *et al.,* 2006). Hence the present study sheds light on genetic diversity present in the super early germplasm the knowledge of which is extremely useful in planning crosses for hybrid and variety development to break the yield barrier in pigeonpea.

Thirty five super early pigeonpea germplasm which are the advanced breeding lines comprising of determinate and non-determinate type was obtained from ICRISAT for assessing genetic diversity using SSR markers (Table 1). Pigeonpea germplasm were grown in pots and leaves from 30 day old seedlings were collected and frozen in liquid nitrogen. 100mg of leaf tissue was ground into fine powder and genomic DNA was extracted following CTAB procedure (Abdelnoor *et al.* 1995). The quality and quantity of DNA of the germplasm were checked through agarose gel electrophoresis (0.8%). A total of 24 SSRs were used for PCR amplification (Table 2). For each SSR marker Polymorphism Information Content (PIC) was determined as described by senior *et al.,* (1998). The amplified PCR products were scored as either presence (1) or absence (0) and entered in the form of a binary matrix. Jaccard's coefficient (J) (Jaccard, 1908) was used to calculate the genetic similarities (GS) based on SSR data.

$$J= N_{11}/ (N_{11}+N_{10}+N_{01})$$

Where N_{11} is the number of bands present in both the individuals; N_{01} is the number of bands present in the individual j, N_{10} is the number of bands present in the individual i and N represents the total number of bands. The similarity matrix was analyzed using NTSYS-pc ver. 2.0 to produce an agglomerative hierarchical classification by employing UPGMA with average linkage. The mean of the similarity matrix would be the cut off line position on the dendrogram to identify the number of clusters. To test the goodness of fit of clustering to a set of data copheneic correlation or cophenetic value was estimated using the COPH and MXCOM options in NTSYS-pc2.02 program.

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Table 1: List of pigeonpea super early germplasm used in the study for assessment of genetic diversity using SSR markers

Genotype	
Non-Determinate type	**Determinate type**
ICPL11276	ICPL11249
ICPL11298	ICPL11263
ICPL11318	ICPL20341
ICPL11300	ICPL11252
ICPL11303	ICPL11255
ICPL11326	ICPL20338
ICPL11279	ICPL20340
ICPL11296	ICPL11253
ICPL11301	ICPL11273
ICPL11242	ICPL20336
ICPL11292	ICPL11256
ICPL11244	ICPL11259
ICPL11245	MN1
ICPL20333	MN5
ICPL11324	
ICPL20335	
ICPL20329	
ICPL20325	
ICPL20327	
ICPL20328	
ICPL20326	

Table 2: Allelic information and Polymorphic information content (PIC) obtained by using SSR markers in Pigeonpea germplasm

Primer	No. of alleles detected	PIC
CCac003	3	0.22
CCttc003	2	0.46
CCttc006	2	0.5
ICPMIE04	4	0.54
PP9	3	0.56
PP5	3	0.56
ASSR-97	3	0.6
Ccac 004	2	0.47
CCac036	3	0.63
CCac011	3	0.69
CCttc002	4	0.48
CCttc004	2	0.53
CCttc008	3	0.35
CCttc007	4	0.63
CCttc033	3	0.59
ICPM1H01	3	0.54
ICPM2B08	4	0.71
PB-1	2	0.17
PP4	4	0.34
PP5	4	0.74
PP8	3	0.63
ICPM1D10	4	0.52
PV14	3	0.59
PV21	3	0.68

Twenty four polymorphic SSR primers were used to analyze the genetic diversity among a set of thirty five super early pigeonpea germplasm comprising of determinate and indeterminate types. These primers resulted in the amplification of 69 bands, with the number of polymorphic bands per primer ranging from 2-4, the average being 2.87. Based on the PIC, SSR primer PP5 was most informative with value of 0.74. The least PIC value of 0.17 was depicted by SSR marker PB-1 (Table 2). Results from AMOVA indicate that approximately 1.1% of genetic diversity was within the gemplasm, 71.3% among germplasm and remaining the 27.5% among the determinate and indeterminate super early germplasm (Table. 3). The dendrogram based on UPGMA clustering clearly divided the thirty five pigeonpea super early germplasm into four distinct clusters (Figure 1). The mean of the similarity matrix data would be the cut off line position on dendrogram. To test the goodness of fit of a clustering to a set of SSR data, cophenetic correlation coefficient or cophenetic value was estimated using the COPH and MXCOMP options in NTSYS-pc program. The cophenetic value of 0.94 obtained using the SSR data indicated a very good fit. Principal Component Analysis revealed that PC1, PC2, PC3 and PC4 accounted for 36%, 19.3%, 5.6% and 4.4% of total variation. Two dimensional and 3-dimensional plots were prepared by using all the four principal components. In the case of 3-D plot based on PCA, four distinct clusters have been identified which is in accordance with UPGMA based clustering (Figure 2). The determinate types and non-determinate types were grouped into separate clusters indicating more genetic similarities within the determinate and non-determinate types (Table 4). It was also reported that use of only few germplasm in pigeonpea breeding program for development of elite cultivars resulted in the narrow genetic base among the released cultivars compared to wild relatives (Kumar *et al*., 2004; Yang *et al*., 2006). In mungbean a set of 615 cultivated, wild and weedy mungbeans were analyzed using simple

Table 3: Analysis of molecular variance of thirty five pigeonpea superearly germplasm using SSR markers

Source	AMOVA for SSR				
	df	SS	MS	Estt Var	%
Between Type	1	1096.015	1096.015	2.719	27.50
Between samples Within Type	32	317.685	9.927	0.116	1.17
Within samples	809	5703.160	7.049	7.049	71.31
Total	842	7116.861	8.452	9.884	100

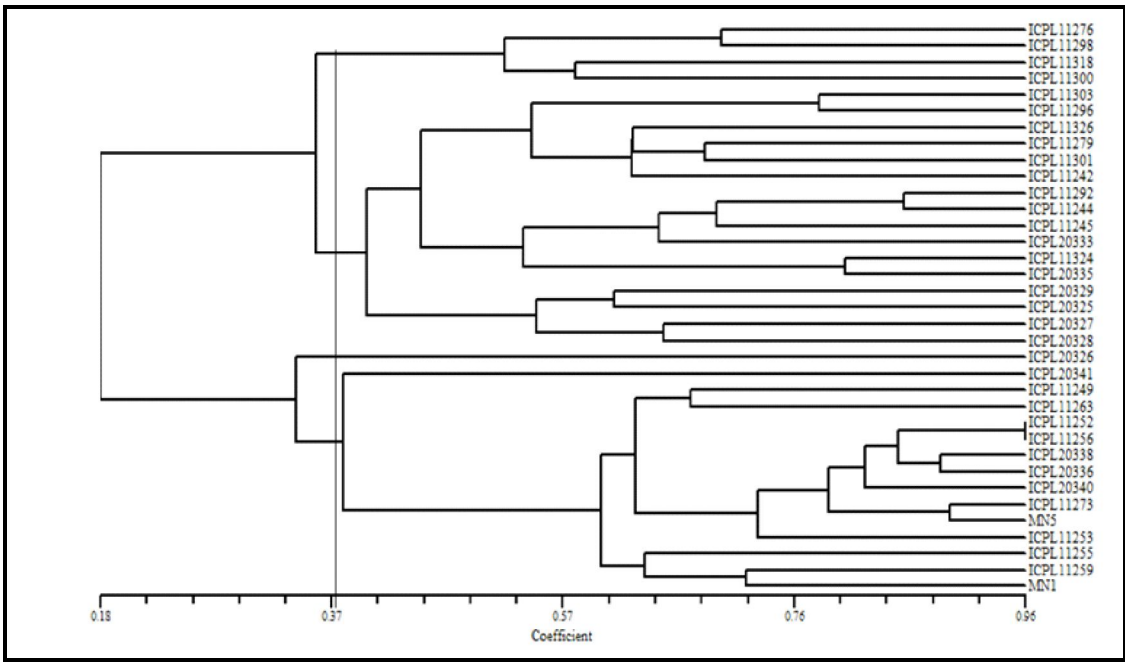

Fig 1: Dendrogram of pigeonpea germplasm based on Jaccard's similarity coefficient of twenty four polymorphic SSR loci

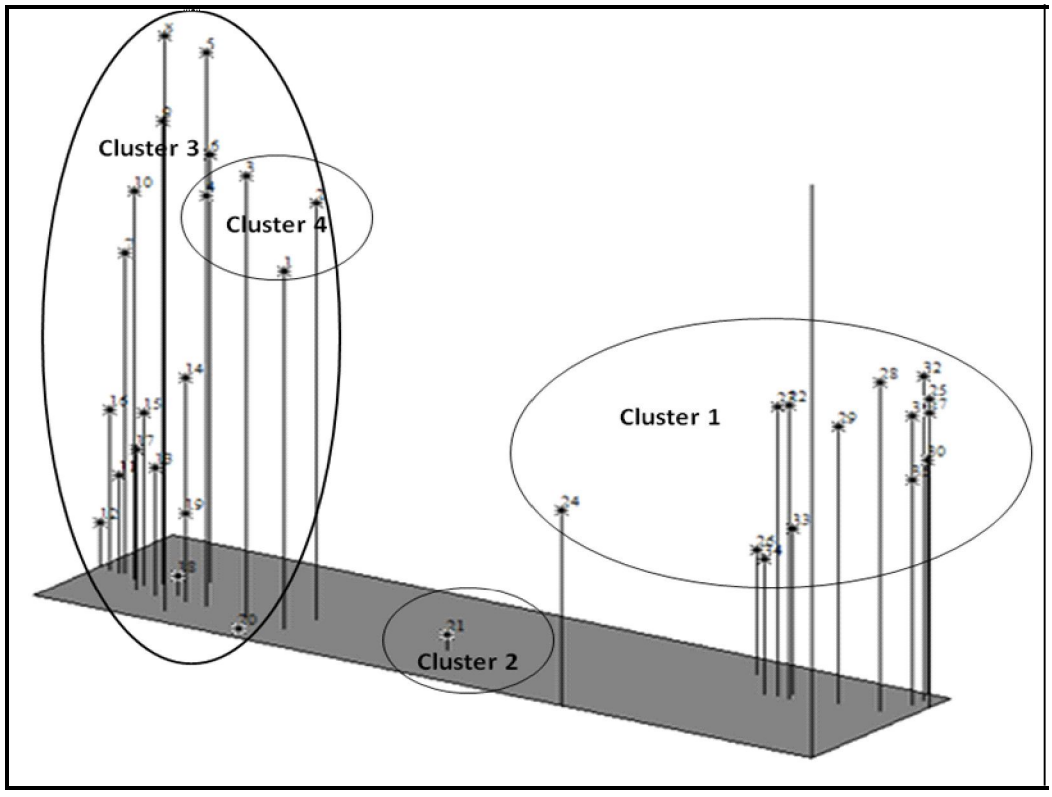

Fig 2: Three dimensional plot of principal components 1, 2, 3 and 4 based on SSR marker data of pigeonpea germplasm

Table 4: Cluster analysis in pigeonpeaSuperearly germplasm based on SSR data

Major Cluster	Sub-cluster	Germplasm
I	A	ICPL 11255, 11259, MN1
	B	ICPL 11252, 20338, 20340, 11252, 11273, 20336, 11256, MN5
	C	ICPL 11249, 11263
	D	ICPL 20341
II	ICPL 20326	
III	A	ICPL 2039, 20325, 20327, 20328
	B	ICPL 20335, 11324, 20333, 11245, 11244, 11292
	C	ICPL 11242, 11301, 11279. 11326, 11296, 11303
IV	A	ICPL 11276, 11298
	B	IPL 11318, 11300

sequence repeat (SSR) markers and found that the markers divided the germplasm into distinct sets according to their genetic distances and origins (Sangiri *et al.*, 2007). Their information can be utilized to compare the performance of hybrids derived from the parents with different degrees of genetic distance. Earlier reports have shown that SSR marker technology could be used to identify heterotic patterns of the parental lines in hybrid rice production (Pirkhezri *et al*., 2007). It is evident from this study that the SSR assay can be useful in pigeonpea breeding programs (Singh *et al*., 2013), for cultivar identification and as well as assessment of the genetic similarity (Odeny *et al*., 2007; Singh *et al*., 2008; Songok *et al*., 2010). The results of the present study confirm that polymorphism based on SSR markers is useful in revealing genetic relatedness between the pigeonpea germplasm under study. Therefore any meaningful effort for construction of genetic relatedness trees can best be done using molecular markers as opposed to agronomic traits which are subject to environmental effects (Varshney *et al*., 2005; Dutta *et al*., 2011: Raju *et al*., 2010). Markers are useful for genotyping accessions and other factors but plant breeders still need the agronomic data to compliment molecular information in order to understand variation among accessions.

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REFERENCES

Abdelnoor, R. V., Barros, E.G. and Moreira, M.A. (1995). Determination of genetic diversity within Brazilian soybean germplasm using random amplified DNA amplification techniques and comparative analysis with pedigree data. *Braz. J. Genet* **18:** 265–273.

Burns, M.J., Edwards, K.J., Newbury, J.H., Ford-Lloyd, V.B. and Baggott, C.D. (2001). Development of Simple Sequence Repeat (SSR) markers for the assessment of genome and genetic diversity in pigeonpea (*Cajanus cajan*). *Mol. Ecol. Notes* **1:** 283-285.

Charcosset, A. and Moreau, L. (2004). Use of molecular markers for the development of new cultivars and the evaluation of genetic diversity. *Euphytica* **137:** 81-94.

Dutta, S., Kumawat, G., Singh, B.P., Gupta, D.K., Singh, S., Dogra, V., Gaikwad, K., Sharma, T.R., Raje, R.S., Bandhopadhya, T.K., Datta, S., Singh, M.N., Bashasab, F., Kulwal, P., Wanjari, K.B., Varshney, R.K., Cook, D.R. and Singh, N.K. (2011). Development of genic-SSR markers by deep transcriptome sequencing in pigeonpea [*Cajanus cajan* (L.) Millspaugh]. *BMC Plant Biology* **11:**17.

Edwards, J., Barker, A., Daly, A., Jones, C. and Karp, A. (1996). Microsatellite libraries enriched for several microsatellite sequences in plants. *Biotechniques* **20:** 758-759.

P. Jaccard. (1908). Nouvelles researches sur ladistribution florale. Bulletin de la Societe Vaudoise des Sciences Naturelles **44:** 223-270.

Kumar, S., Gupta, S., Chandra, S. and Singh, B.B. (2004). How wide is the genetic base of pulse crops. In Pulses in new perspective (ed. M. Ali, B.B. Singh, S. Kumar and Vishwadhar). IIPR, Kanpur: 211-221.

Odeny, D.A., Jayshree, B., Ferguson, M., Hoisington, D., Crouch, J. and Gebhardt, C. (2007). Development, Characterization and Utilization of Microsatellite Markers in Pigeonpea. *Plant Breeding* **126:** 130-136.

Panguluri, S. K., Janaiah, K., Govil, J.N., Kumar, N., P. A. and Sharma, P.C. (2006). AFLP fingerprinting in pigeonpea [*Cajanus cajan* (L.) Millsp.] and its wild relatives. *Genet. Res. Crop Evo* **53:** 523–531.

Pirkhezri, M., Hassani, M.E. and Hadian, J. (2010). Genetic diversity in different populations of Matricaria chamomilla L. growing in Southwest of Iran, based on morphological and RAPD markers. *Research Journal of Medicinal Plant* **4:** 1-13.

Raju, N.L., Gnanesh, B.N., Lekha, P., Jayashree, B., Pande, S., Hiremath, P.J., Byregowda, M., Singh, N.K. and Varshney, R.K. (2010). The first set of EST resource for gene discovery and marker development in pigeonpea (*Cajanus cajan* L.). *BMC Plant Biology* **10:** 45.

Ratnaparkhe, M.B., Gupta, V.S., Murthy, M.R. and Ranjekar, P.K. (1995). Genetic Fingerprinting of Pigeonpea [Cajanus cajan (L.) Millsp.] and Its Wild Relatives Using RAPD Markers. *Theor. Appl. Genet* **91:** 893-898.

Sangiri, C., Kaga, A., Tomooka, N., Vaughan, D.A. and Srinives, P. (2007). Genetic diversity of the mungbean (Vigna radiata, Leguminosae) gene pool based on microsatellite analysis. Australian. *Journal. of Botany* **55:** 837-847.

Senior, M.L., Murphy, J.P., Goodman, M.M. and Stuber, C.W. (1998). Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. *Crop Science* **38:** 1088-1098.

Singh, A.K., Rai, V.P., Chand, R., Singh, R.P. and Singh, M.N. (2013). Genetic diversity studies and identification of SSR markers associated with Fusarium wilt (*Fusarium udum*) resistance in cultivated pigeonpea (*Cajanus cajan*). *Journal of Genetics* **92:** 273-280.

Singh, N. K., Gupta, D.K., Jayaswal, P.K., Mahato, A.K., Dutta, S., Singh, S. *et al*. (2012). The first draft of the pigeonpea genome sequence. *J. Pl. Biochem. Biotech* **21:** 98–112.

Singh, S., Singh, K.N., Kant, R., Mehfooz, S. and Dutta, S. (2008). Assessment o genetic diversity among pigeonpea genotypes using SSR markers. *Indian J. Genet* **68:** 255-260.

Sivaramakrishnan, S., S.L.J. Kannan..J. and Reddy. (2002). Diversity in selected wild and cultivated species of pigeonpea using RFLP of mtDNA. *Euphytica* **125:** 121-128.

Songok, S., Ferguson, M., Muigai, A.W. and Silim, S. (2010). Genetic diversity in pigeonpea [Cajanus cajan (L.) Millsp.] Landraces as revealed by simple sequence repeat markers. *African Journal of Biotechnology* **9:** 3231-3241.

Varshney, R. K., Graner, A. and Sorrells, M. E. (2005). Genic microsatellite markers in plants: features and applications. *Trends Biotechnol* **23:**48-55.

Varshney, R. K., Chen, W., Li, Y., Bharti, A.K., Saxena, R.K., Schlueter, J.A. *et al*. (2012). Draft genome sequence of pigeonpea (*Cajanus cajan*), an orphan legume crop of resource-poor farmers. *Nat. Biotech*. 30: 83–89.

Yang, S., Pang, W., Ash, G., Harper, J., Carling, J., Wenzl, P., Huttner, E., Zong, X. and Kilian, A. (2006). Low level of genetic diversity in cultivated Pigeonpea compared to its wild relatives is revealed by diversity arrays technology. *Theor. Appl. Genet* **113:** 585-595.