Indian J. Genet., 69(1): 35-43 (2009)

Microsatellite DNA marker aided diversity analysis in cowpea [*Vigna unguiculata* (L.) Walp]

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(Received: November 2008; Revised: January 2009; Accepted: February 2009)

Abstract

Eighty three cowpea genotypes were analyzed with 15 micro satellite markers to test the genetic variation. Among the total 15 SSR primers used, 12 primers were polymorphic and 3 primers were monomorphic. A total of 15 markers were obtained, among which 80% were polymorphic and 20% were monomorphic. Genotype specific markers were identified for some genotypes. The clusters constructed based on SSR marker data revealed significant genetic variation among the genotypes. The marker detected significant polymorphism among the local landraces as compared to the cultivated varieties. Genotypes with resistance to rust disease and nutritionally superior ones grouped together in separate clusters. The results using SSR markers indicated that micro satellites successfully unraveled the genetic variation existing in selected cowpea genotypes.

Key words: Cowpea, microsatellite, polymorphism, dendrogram, germplasm, breeding

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is an important grain legume crop grown for its protein rich grains. It is an inexpensive source of protein, vitamins and minerals, and plays an important role in human consumption and animal feeding in developing countries of Africa, Asia and Latin America. Development of new cultivars with early maturity, acceptable grain quality, resistance to some important diseases and pests has significantly increased the yield of cowpea [1]. Loss of genetic diversity, in part due to the conventional breeding selection programs associated with modern agricultural practices, has genetically eroded for many cultivated species. Better knowledge of the genetic similarity of breeding materials could help to maintain genetic diversity and sustain long-term selection gain by incorporating in breeding programmes. Furthermore, monitoring the genetic variability within the gene pool of elite breeding material would make crop improvement more efficient. Little information is available about the extent of genetic diversity among cowpea landrace/ genotypes for long term conservation and improvement.

Only few genetic studies on cowpea using molecular marker techniques (RAPD, RFLP, AFLP, SSR) have been reported [2]. The genetic similarity in cultivated cowpea has been assessed on the basis of morphological and physiological traits [3] allozymes [4, 5] seed storage proteins [6], chloroplast DNA polymorphism [7], restriction fragment length polymorphisms (RFLP) [8], amplified fragment length polymorphisms (AFLP) [9] and random amplified polymorphic DNA (RAPD) [10]. However these reports fell short of providing quantification of genetic variability within species, especially regarding genetic distances.

Among DNA based approaches for crop improvement, the first step of molecular breeding is the use of molecular markers as a tool to detect the extent and structure of genetic variation, providing insights into the diversity of crop varieties and potential contributions represented by their wild relatives. Microsatellite markers have become the DNA markers of choice for a wide range of applications in genetic mapping and genome analysis genotype identification and variety protection [12] seed purity evaluation and germplasm conservation, diversity studies [13] paternity

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42	EC394745	Exotic, collection from BARC, Mumbai	HS to rust & YMV
43	IC204103	Cultivar,Bihar	DR, low yielder
44	EC 324858	Exotic, collection from BARC, Mumbai	High yielding & promising
45	IC 219594	Land race from Andra Pradesh	Low yielder
46	IC 202782	Land race, from Goa	DR, high yielding & promising
47	EC 394805	Exotic, collection from BARC, Mumbai	HS to rust
48	IL-4	Introgressed line, UAS, Dharwad	Promising & indeterminate
49	IC 253270	Cultivar, Andra Pradesh	DR, low yielder
50	IC 202841	Local cultivar, Orissa	HS to rust & YMV
51	IC 202743	Land race from Andra Pradesh	HS to rust
52	EC 394823	Exotic collection from BARC, Mumbai	HS to rust
53	IC 202797	Landrace, South Goa	Bold seeded, long pods & DR
54	DCS-6	Mutant of KBC-1, UAS, Dharwad	DR & promising
55	IC 214835	Land race, Andra Pradesh	HS to rust
56	IC 253255	Origin not known, collection from NBPGR	Resistant to rust & poor yielder
57	IC 202779	Landrace, Goa	Long pods, bold seeds & vegetable type
58	IC 253281	Origin not known, New Delhi	Rust resistant & low yielder
59	IC 202860	Landrace, Orissa	Rust resistant & low yielder
60	IC 202707	Land race, Uttara Pradesh	HS to rust
61	IC 259085	Landrace, Kerala	HS to rust
62	IC 214833	Land race from Andra Pradesh	DR, low yielder
63	IL-3	Introgressed line, UAS, Dharwad	DR, promising & high yielding
64	EC 394753	Exotic collection from BARC,Mumbai	Promising & high yielding
65	IC 214752	Land race from Andra Pradesh	HS to rust
66	IC 257952	Origin not known, collection from NBPGR	Susceptible to rust
67	DCS-5	Mutant of KBC-1, UAS, Dharwad	DR, determinate & promising
68	IC 219607	Land race, Andra Pradesh	HS to rust
69	IC 253275	Origin not known, New Delhi	Resistant to rust
70	IC 97806	Origin not known, collection from NBPGR	Resistant to rust
71	IC 202868	Cultivar, Orissa	Resistant to rust
72	IC 219872	Land race from Andra Pradesh	DR, low yielder
73	IC 214836	Land race from Andra Pradesh	Promising & rich in nutrients
74	IL-2	Introgressed line, UAS, Dharwad	Bold seeded, indeterminate & DR
75	T-2	Back cross derivative, UAS,Dharwad	Early, promising & determinate
76	IC 243353	Land race from Andra Pradesh	Rust resistant
77	IC 249593	Land race from Andra Pradesh	HS to rust
78	GC 3	Released Variety	HS to YMV
79	IC 97767	Origin not known, collection from NBPGR	DR & promising, high yielding
80	EC 394767	Exotic, collection from BARC, Mumbai	High yielding
81	M-23	Mutant of C-152, UAS, Dharwad	DR & promising, high yielding
82	IC 68786	Origin not known, collection from NBPGR	DR, high phosphorus
83	IC 202784	Landrace, South Goa	High protein & nutrients & DR

DR : Disease resistant to rust, bacterial blight and powdery mildew; HS : Highly susceptible; R : Resistant; YMV : Yellow Mosaic Virus

could be because of variation in copy number of amplified sequences. In earlier reports, this primer also amplified breeding lines from IITA and one wild cowpea genotype [19]. These results suggest that the flanking regions of VM 22 primer pairs are conserved in *Vigna unguiculata* but the number of copies varies among cowpea genotypes. This is in support with the earlier findings in cowpea [18]. A relatively high level of variation was found in the cowpea germplasm using SSR markers, where in more than 75% bands were polymorphic. These results are in agreement with SSR study on cowpea [19] indicated that sequence variations does exists in cowpea.

A dendrogram was constructed with genotypic data from 12 polymorphic loci clearly formed two major clusters at 85% dissimilarity (Fig. 3). Genotypes IC-202868, IL-3, IC-214835, EC-394767, IC-97767, GC-3, IC-249593, IC- 243353, T-2, IC-253275, IC-202784, IC-68786, IC-202860, IC-253281, M-23, IC-202779 and IL-4 (17) formed one cluster and remaining 66 genotypes formed another cluster. Accessions with close geographic origin (IC 219872 & IC 214836, IC 243353 & IC249593) or pedigree background (IL 3, T2 & IL 4) clustered together. However the 66 genotypes at 55 % dissimilarity divided into two sub groups. Three genotypes, IL-2, IC-97806 and EC-394753 formed one separate cluster. The main clusters with 63 accessions were divided into 2 subgroups/clusters at 30 % dissimilarity level (50 and 13 genotypes). This shows the close origin and distribution of these cowpea accessions.

Dendrogram indicated clear pattern of clustering according to the geographical location in which they were collected. Similar observations were made by earlier workers in cowpea [20]. Based on environmental adaptation, the genotypes were clustered in to different groups and their close association with a set of markers depicted in Fig. 4. The exact marker data revealed that VM 39 exhibited more heterozygous loci and closely associated with landraces of Goa. The backcross derivatives of common pedigree were clustered in a group based on genotypic data of SSR loci and their close association with markers VM 35 and VM 19. The marker (VM19) association is distinct with two

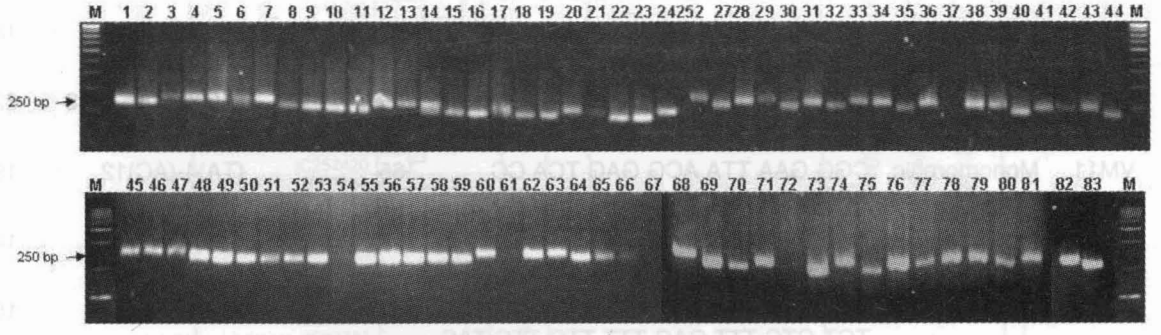

Fig. 1A&B. Polymorphic banding pattern of 83 genotypes using SSR primer VM-68

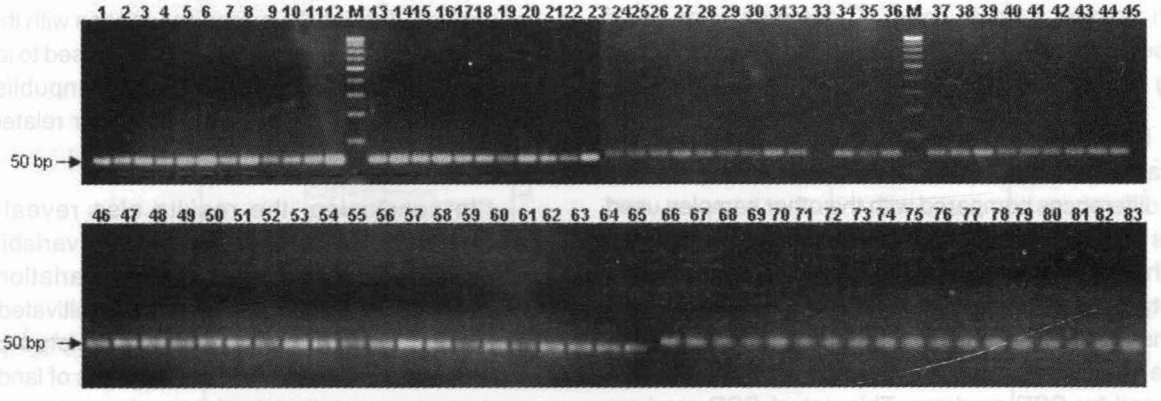

Fig. 2A&B. Monomorphic banding pattern of 83 genotypes using SSR primer VM-17

Table 2. List of SSR primers used in the present study

S.No.	Primer name	Nature of amplification	Sequence (5'-3')	Annealing temperature	Nucleotide repeats	Size of amplified fragments
1	VM5	Polymorphic	AGC GAC GGC AAC AAC GAT TTC CCT GCA ACA AAA ATA CA	63	(AG)32	188
2	VM19	Polymorphic	TAT TCA TGC GCC GTG ACA CTA TCG TGG CAC CCC CTA TC	65	(AC)7-(AC)5	241
3	VM22	Polymorphic	GCG GGT AGT GTA TAC AAT TTG GTA CTG TTC CAT GGA AGA TCT	57.8	(AG)12	217
4	VM25	Polymorphic	CCA CAA TCA CCG ATG TCC AA CAA TTC CAC TGC GGG ACA TAA	63	(TC)18	240
5	VM31	Polymorphic	CGC TCT TCG TTG ATG GTT ATG GTG TTC TAG AGG GTG TGA TGG TA	60	(CT)16	200
6	VM35	Polymorphic	GGT CAA TAG AAT AAT GGA AAG TGT ATG GCT GAA ATA GGT GTC TGA	59.55	(AG)11.(T)9	127
7	VM36	Polymorphic	ACT TTC TGT TTT ACT CGA CAA CTC GTC GCT GGG GGT GGC TTA TT	64	(CT)13	160
8	VM37	Polymorphic	TGT CCG CGT TCT ATA AAT CAG C CGA GGA TGA AGT AAC AGA TGA TC	63.1	(AG)5.(CCT) 3.(CT)13	289
9	VM39	Polymorphic	GAT GGT TGT AAT GGG AGA GTC AAA AGG ATG AAA TTA GGA GAG CA	60.75	(AC)13.(AT) 5.(TACA)4	212
10	VM68	Polymorphic	CAA GGC ATG GAA AGA AGT AAG AT TCG AAG CAA CAA ATG GTC ACA C	60	(GA)15	254
11	VM70	Polymorphic	AAA ATC GGG GAA GGA AAC C GAA GGC AAA ATA CAT GGA GTC AC	59.55	(AG)20	186
12	VM71	Polymorphic	TCG TGG CAG AGA ATC AAA GAC AC TGG GTG GAG GCA AAA ACA AAA C	68.1	(AG)12.(AAAG)3	225
13	VM11	Monomorphic	CGG GAA TTA ACG GAG TCA CC CCC AGA GGC CGC TAT TAC AC	65	(TA)4-(AC)12	195
14	VM14	Monomorphic	AAT TCG TGG CAT AGT CAC AAG AGA ATA AAG GAG GGC ATA GGG AGG TAT	65	(AG)24	144
15	VM17	Monomorphic	GGC CTA TAA ATT AAC CCA GTC T TGT GTC TTT GAG TTT TTG TTC TAC	60	(CT)12	152

nutritionally rich landraces IC 202784 and IC 68786, which formed a single group. These genotypes could be used efficiently in the nutrition breeding programme using marker assisted selection.

Preliminary phenotypic data of these cowpea landraces/genotypes (data not shown) [21, 22] showed wide differences compared with the other samples used in this study. The disease resistant genotypes clustered together in one group, whereas the highly susceptible genotypes were grouped in a single cluster (Fig. 4). This means that, there could be some similarities between these accessions at DNA level and their linkage exhibited by SSR markers. This set of SSR markers (VM39, VM-68, VM35, VM19, VM5, VM22) showed distinct bands in disease resistance and susceptible genotypes indicating their close association with the trait. These germplasm lines are being further used to identify differential races of cowpea rust [28 unpublished]. Further, future studies could confirm their relatedness in this direction.

In conclusion, the results also reveal wide polymorphism indicating the available variability at genetic level. Higher polymorphism/variation was observed in local landraces rather than cultivated ones because of broad genetic base as depicted by the markers used. Therefore, the useful genes of landraces needs to be exploited further and can be used in breeding programmes. The genetic diversity obtained

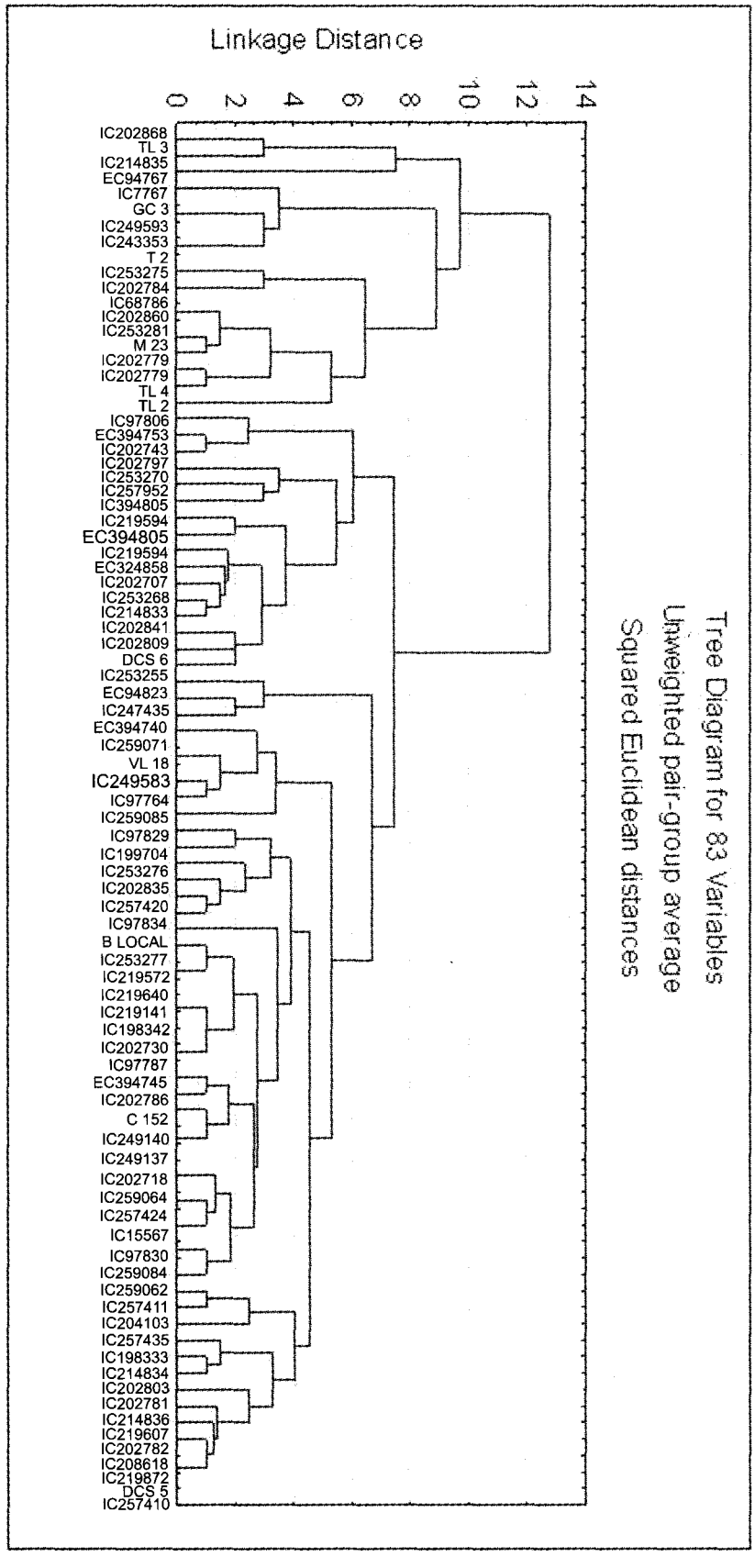

Fig. 3. Dendrogram constructed for 83 cowpea accessions with genotypic data

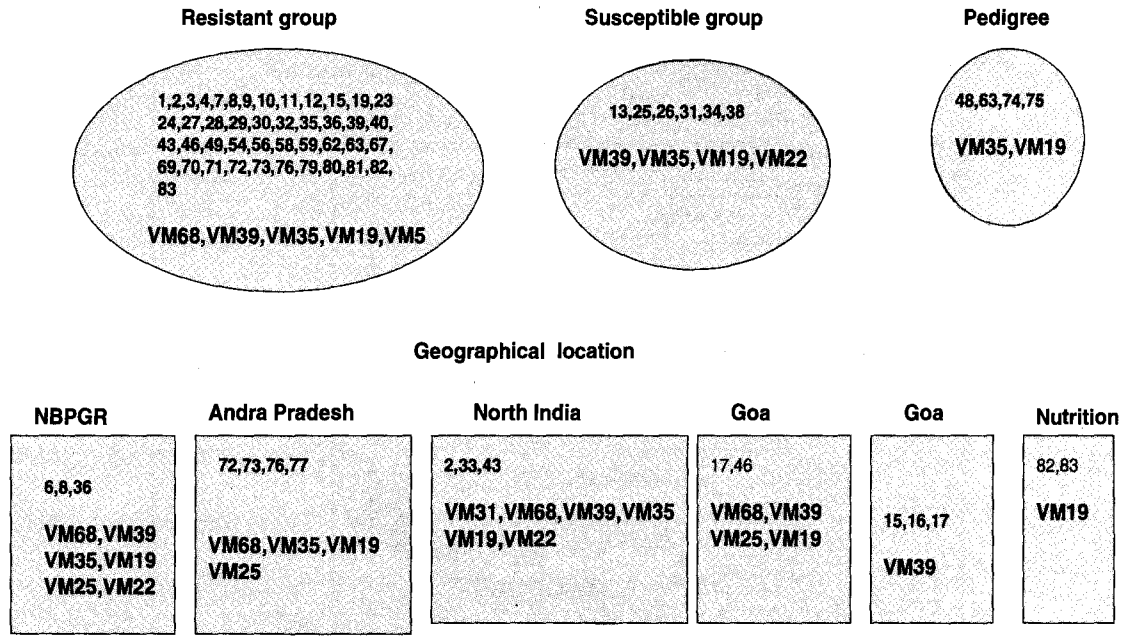

Fig. 4. Marker association with different groups of genotypes. The number plotted represents individual cultivars and corresponds to the ones listed in Table 1

in this study might be useful in future strategies for evaluation of desired genotypes. Such molecular data would be also useful for detecting DNA patterns unique for a given accession or set of accessions. Finally, our results demonstrate the feasibility of the SSR markers for quantifying genetic distances among 83 cowpea landraces/genotypes.

Acknowledgements

The authors are gratefully thank GCP-SP 5 for providing minigrant support to conduct this research work. We also thank University of Agricultural Sciences, GKVK, Bangalore for providing financial assistance to purchase consumables. We also thank NBPGR, New Delhi for supplying the cowpea seed material.

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