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Research Note

Molecular characterization of pigeonpea (*Cajanus cajan* (L.) Millsp.) germplasm by microsatellite markers

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Abstract
Ten pigeonpea specific SSR markers were used to test the molecular diversity among 60 pigeonpea accessions. Six markers were polymorphic and four of them were monomorphic. Totally 22 alleles were produced by six polymorphic markers. The PIC value of the markers ranged from 0.033(CcM1381) to 0.654 (CcM2977).The average PIC value of the markers was 0.205. Based on the dendrogram constructed using the dissimilarity values, 60 accessions were grouped into three main clusters. Most of the accessions were found to be present in main cluster II. Cluster III depicts that one one accession Pusa 992 forms solitary cluster. Based on dendrogram and neighbor joining Pusa 992 forms separate cluster in both these types. There is no significant molecular diversity among the cultivated genotypes. Such a narrow genetic base is likely to represent a serious impediment to breeding programs in pigeonpea. This study suggested the need for broadening the genetic base of cultivated genotypes to improve the productivity of the crop.

Key words
Redgram germplasm, microsatellite markers, polymorphism

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a grain legume crop of the tropics and subtropics for its high protein (18 to 22%) seeds particularly in the Indian subcontinent where it accounts for 70% of the world's production and coverage. Analyzing genetic relationships in species is important for revealing diversity. In addition to displaying the existing variability among cultivars genetic diversity provides valuable information on target trait availability and diversity for successful breeding programs. Molecular marker studies are increasingly important tools for genetic and genomic studies, breeding and biodiversity research. Currently several DNA-based molecular marker technologies are available for genetic diversity analysis. Among different types of molecular markers, markers based on simple sequence repeats (SSR) had been shown to be highly polymorphic even between closely related individuals within a species (Edwards *et al*., 1996) and tend to show more polymorphism than many alternative marker systems (Doldi *et al*., 1997). Microsatellites have been increasingly used to assess genetic diversity and population structure among plants (Li *et al*., 2000 and Pillen *et al*., 2000).

Plant materials: Totally 60 cultivated pigeonpea genotypes of different geographical origin were used for this study (Table 1). The seed materials for all the accessions were obtained from Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore.

DNA extraction and PCR analysis: DNA was extracted from young fresh leaves of pigeonpea. The DNA was extracted using mini CTAB method. DNA quality from all the individual samples was checked through 0.8% Agarose gel electrophoresis before PCR amplification. A total of 10 SSR markers were selected for PCR analysis based on their performance and reproducibility (Table 2). PCR analysis was performed in a 10µl reaction volume containing 50 ng of genomic DNA, 0.65 µl of each forward and reverse markers (5µM), 0.4 µl of 10mM dNTPs, 0.2 µl of Taq polymerase and 1.0 µl of 10X PCR assay Buffer. The PCR conditions include, initial denaturation at 94ºC for 3 minutes followed by 35 cycles, each consisting of denaturation at 94ºC for 1minute, annealing at appropriate temperature for 1 min. and elongation at 72ºC for 2 min. A final extension included 72ºC incubation for 10 minutes followed by a hold at 4ºC. Master Cycler Gradient PCR (Biorade) was used to carry out the reaction. The amplified DNA fragments were observed in Ethidium bromide stained 2% agarose gel in 1X TBE buffer at 120 V. The gels were visualized on trans UV illuminator and photographed in Gelstan gel documentation system.

Statistical analysis: The amplicons were scored and the data were given by their allelic sizes as allelic data. The scores were obtained in the form of a matrix with '1' or '0', which indicated the presence or absence of bands in each species respectively. The binary data score were used to construct a dendrogram. Using the DARwin 6.0 software package (Perrier and Jacquemond- Collet, 2014) a simple matching dissimilarity index was calculated from the allelic size data set and the matrix was then subjected to UPGMA and Neighbour-joining analysis.

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Dissimilarity between units I and j, $dij = 1 - 1/L \sum_{l=1}^{L} (ml/\pi)$

where, L = number of loci; π = ploidy;ml = number of matching alleles for locus l.

Cluster analysis was performed using UPGMA strategy to obtain a dendrogram. Neighbour-joining tree was also developed based on unweighted average for dissimilarity index. Markers were described according to the number of alleles and their Polymorphic Information Content (PIC). PIC is a measure of the informativeness of a genetic marker in any species (Botstein *et al.*, 1980*;* Liu *et al.*, 2000). Polymorphism information content (PIC) or expected heterozygosity score for each SSR marker was calculated based on the formula.

$$PIC, Hj = 1-\sum pi^2$$

where Pi is the frequency for the i^{th} allele (Nei, 1973).

Totally 10 primers were randomly selected and used to check amplification among the 60 accessions of pigeonpea. Of the 10 SSR primers evaluated among 60 accessions, six primers produced clear, scorable and polymorphic marker profiles and were used for further analysis and four among them were monomorphic. A total of 22 alleles were detected and the number of alleles detected per primer pair ranged from 2 to 6 with an average 2.2 alleles per locus. The polymorphic information content (PIC) value ranges from 0.033 (CcM1381) to 0.654 (CcM2977). The average PIC value of polymorphic markers was observed to be 0.205 (Table 2). The maximum number of six amplified products was observed in the profiles of primer CcM2977. Hence this primer is very much useful for molecular diversity studies.

Based on the polymorphic SSR markers, the 60 accessions were grouped into three clusters as given (Fig. 1.) to find the genetic distance among the pigeonpea accessions. The dendrogram showed three main clusters, indicating Pusa 992 as a separate cluster (main cluster III) with 18 accessions in main cluster I and rest of the accessions (41) in main cluster II. Cluster II was the largest one containing many sub clusters. The dendrogram (Fig. 1) and Neighbour- joining tree (Fig. 2) depicts that the accession Pusa 992 forms separate solitary cluster. So this accession will be useful for crossing or any breeding programme. Although ample morphological diversity is exhibited by pigeonpea, but the same is not true at the molecular level (Yang *et al.*, 2006). Hence, this study strongly recommends the need for broadening the genetic base of the cultivated pigeonpea crop by attempting crosses with high molecular diversity could be used in breeding to broaden the genetic base of pigeonpea.

References

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am.J.Hum.Genet.* **32**: 314-331.

Doldi, M.L., Vollmann, J. and Lelly, T. 1997. Genetic diversity in soyabean as determined by RAPD and microsatellite analysis. *Plant Breed.,* **116**: 331-335.

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

Li, C.D., Rossnagel, B.G. and Scoles, G.J. 2000. The development of oat microsatellite markers and their use in identifying relationships among *Avena* species and oat cultivars. *Theor. Appl. Genet.*, **101**: 1259-1268.

Liu, S., Cantrell, R.G., McCary, J.C. and Sterwart, J. 2000. Simple sequence based assessment of genetic diversity in cotton race stock accessions. *Crop Sci.*, **40**: 1459-1469

Nei, M. 1973. The theory and estimation of genetic distance. *In*: N.E. Morton (Eds). Genetic Structure of Populations, University of Hawaii Press, Honolulu, pp.45-54.

Perrier, X. and Jacquemoud-Collet, J.P. 2014. DARwin software, version 6. http://darwin.cirad.fr/darwin.

Pillen, K., Binder, A., Kreuzkam, B., Ramsay, L., Augh, R.W., Forster, J. and Leon, J. 2000. Mapping new EMBL derived barley microsatellites and their use in differentiating German barley cultivars. *Theor. Appl. Genet.*, **101**: 652-660.

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.

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Table 1. Details of Pigeonpea accessions, their source, origin and some specific characteristics

S. No.	Genotypes	Origin	Growth habit with resistance to stress
1.	ICP 245335	Andhra Pradesh	Indeterminate, Moderately susceptible to wilt, SMD
2.	ICP 245375	Andhra Pradesh	Indeterminate, Moderately susceptible to wilt, SMD
3.	ICP 245474	Andhra Pradesh	Indeterminate, Moderately susceptible to wilt, SMD
4.	ICP 245517	Andhra Pradesh	Indeterminate, Moderately susceptible to wilt, SMD
5.	ICP 245527	Andhra Pradesh	Indeterminate, Moderately susceptible to wilt, SMD
6.	ICP 245532	Andhra Pradesh	Indeterminate, Moderately susceptible to wilt, SMD
7.	ICP 245534	Andhra Pradesh	Indeterminate, Moderately susceptible to wilt, SMD
8.	ICP 245541	Andhra Pradesh	Indeterminate, Moderately susceptible to wilt, SMD
9.	ICP 8863	Andhra Pradesh	Indeterminate, Resistant Fusarium wilt,
10.	ICP 9174	Andhra Pradesh	Indeterminate, Resistant Fusarium wilt,
11.	AL 601	PAU, Ludhiana	Indeterminate, Susceptible to wilt, SMD
12.	AL 611/2	PAU, Ludhiana	Indeterminate, Susceptible to wilt, SMD
13.	CRG 9060	TNAU, Coimbatore	Indeterminate, Moderately Resistant to SMD
14.	WRG 27	Andhra Pradesh	Indeterminate, Susceptible to wilt, SMD
15.	ICP15874	*Cajanus cinereus*	Indeterminate, disease reaction not scored
16.	IC 15782	*Dimbaria ferruginea*	Indeterminate, disease reaction not scored
17.	Pusa 992	IARI, New Delhi	Indeterminate, Resistant to PSB and SMD
18.	Pusa 2002	IARI, New Delhi	Indeterminate, Tall, Semi spreading, early maturity
19.	IC 525519	Andhra Pradesh	Indeterminate, Moderately susceptible to wilt, SMD
20.	Pusa 992	IARI, New Delhi	Indeterminate, Resistant to PSB and SMD
21.	ICPL 86020	ICRISAT	Indeterminate, Moderately tolerant to SMD
22.	CRG 5/6	TNAU, Coimbatore	Indeterminate, Moderately Resistant to SMD
23.	ICPR 2431	ICRISAT	Indeterminate, Moderately Resistant to SMD
24.	ICPL 2438	ICRISAT	Indeterminate, Moderately Resistant to SMD
25.	ICPR 2363	ICRISAT	Indeterminate, Moderately Resistant to SMD
26.	ICPR 2447	ICRISAT	Indeterminate, Moderately Resistant to SMD
27.	ICPR 525585	ICRISAT	Indeterminate, Moderately Resistant to SMD
28.	DT 2	IARI, New Delhi	Indeterminate, Drought tolerant
29.	IC 525468	ICRISAT	Indeterminate, Moderately Resistant to SMD
30.	ICP 1	ICRISAT	Indeterminate, Moderately Resistant to SMD
31.	ICP 2387	ICRISAT	Indeterminate, Moderately Resistant to SMD
32.	C 2291	Andhra Pradesh	Indeterminate, Tolerant to Fusarium wilt
33.	C11	Andhra Pradesh	Indeterminate, Tolerant to Fusarium wilt
34.	IC 73999	ICRISAT	Indeterminate, Moderately Resistant to SMD
35.	KPL 43	Kanpur, UP	Indeterminate, Moderately Resistant to SMD
36.	KPL 44	Kanpur, UP	Indeterminate, Moderately Resistant to SMD
37.	IPA 38A	Kanpur, UP	Indeterminate, Resistant Fusarium wilt,
38.	BDN 2	Maharashtra	Indeterminate, Tolerant to Fusarium wilt,
39.	Bahar	Bihar	Indeterminate, Resistant to SMD
40.	ICPL 81-3	ICRISAT	Indeterminate, Moderately Resistant to SMD
41.	ICPL 89	ICRISAT	Indeterminate, Moderately Resistant to SMD
42.	ICPL 149	ICRISAT	Indeterminate, Moderately Resistant to SMD

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Table 1. Contd.,

S. No.	Genotypes	Origin	Growth habit with resistance to stress
43.	CO5	TNAU, Coimbatore	Indeterminate, Susceptible to SMD
44.	ICPL 87091	ICRISAT	Indeterminate, Moderately Resistant to SMD
45.	ICPL 90047	ICRISAT	Indeterminate, Moderately Resistant to SMD
46.	ICPL 90047	ICRISAT	Indeterminate, Moderately Resistant to SMD
47.	ICPL 92047	ICRISAT	Indeterminate, Moderately Resistant to SMD
48.	UPAS 120	Pantnagar	Suitable for double cropping in wheat, susceptible to SMD
49.	ICP 7234	ICRISAT	Indeterminate, Moderately Resistant to SMD
50.	ICP 10697	ICRISAT	Indeterminate, Moderately Resistant to SMD
51.	IC 73895	ICRISAT	Indeterminate, Moderately Resistant to SMD
52.	IC 332084	ICRISAT	Indeterminate, Moderately Resistant to SMD
53.	IC 342747	ICRISAT	Indeterminate, Moderately Resistant to SMD
54.	IC 339057	ICRISAT	Indeterminate, Moderately Resistant to SMD
55.	IC 123325	ICRISAT	Indeterminate, Moderately Resistant to SMD
56.	AL 1692	PAU, Ludhiana	Indeterminate, Susceptible to wilt, SMD
57.	AL 1727	PAU, Ludhiana	Indeterminate, Susceptible to wilt, SMD
58.	AL 1730	PAU, Ludhiana	Indeterminate, Susceptible to wilt, SMD
59.	AL 1733	PAU, Ludhiana	Indeterminate, Susceptible to wilt, SMD
60.	AL 1734	PAU, Ludhiana	Indeterminate, Susceptible to wilt, SMD

Table 2. List of primers used for this present study

Marker	Sequences	No. of alleles detected	PIC value	Allele size range (bp)	Annealing temperature ^{o}C
CcM 2977	F: TTGATTTGAGTCTGCCCAATC R: AAAAGCTCCAACGTGTGTCC	6	0.654	160-270	59.50
CcM0039	F: AGGAATAATGTTTGCTGCGG R: TTGGTATGTGGAACGATTGC	4	0.519	120-400	59.00
CcM1381	F:AGCACACAGTACGGAAAGCTC R:GGCAAATGTTTTCTCTGGAATC	2	0.033	150-200	63.00
CcM0252	F: CATAGAAGCCCACCTTCCAA R:CTGCATGCAAAACGAAGAAG	2	0.183	140-160	58.00
CcM0268	F:CCTTTTGGGTTAGGGTATCCA R:CCCCTAACGTAGCCTGTCAA	2	0.180	190-200	60.00
CcM1538	F:AACAACAAACAAGCAAGGGC R:TCAAGTAAATGAATAGCTCATCGAA	2	0.485	250-255	64.00
CcM0008	F:CGGTGAAAAGGGTCAATGAG R:CAAAATTAAAGCCTACTTATTTTACGA	1	0.000	200	58.00
CcM0093	F:TCATTGACCCCTCTGGAAAT R:ACAATTGGAAAAATAAGTGAGTGAT	1	0.000	250	58.00
CcM0306	F:TGTTCCCAAGGTTATCGACC R:GCCTGCATCCTTTTGTAGTTG	1	0.000	250	60.00
CcM0353	F:GATTCGCAAGTTGCTCCTTC R: TTGTGATCACTTATCATCATTTTG	1	0.000	200	61.00
	Total	**22**	**-**		
	Mean	**2.2**	**0.205**		

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Fig. 1. UPGMA dendrogram showing the clustering of 60 accessions of Pigeonpea

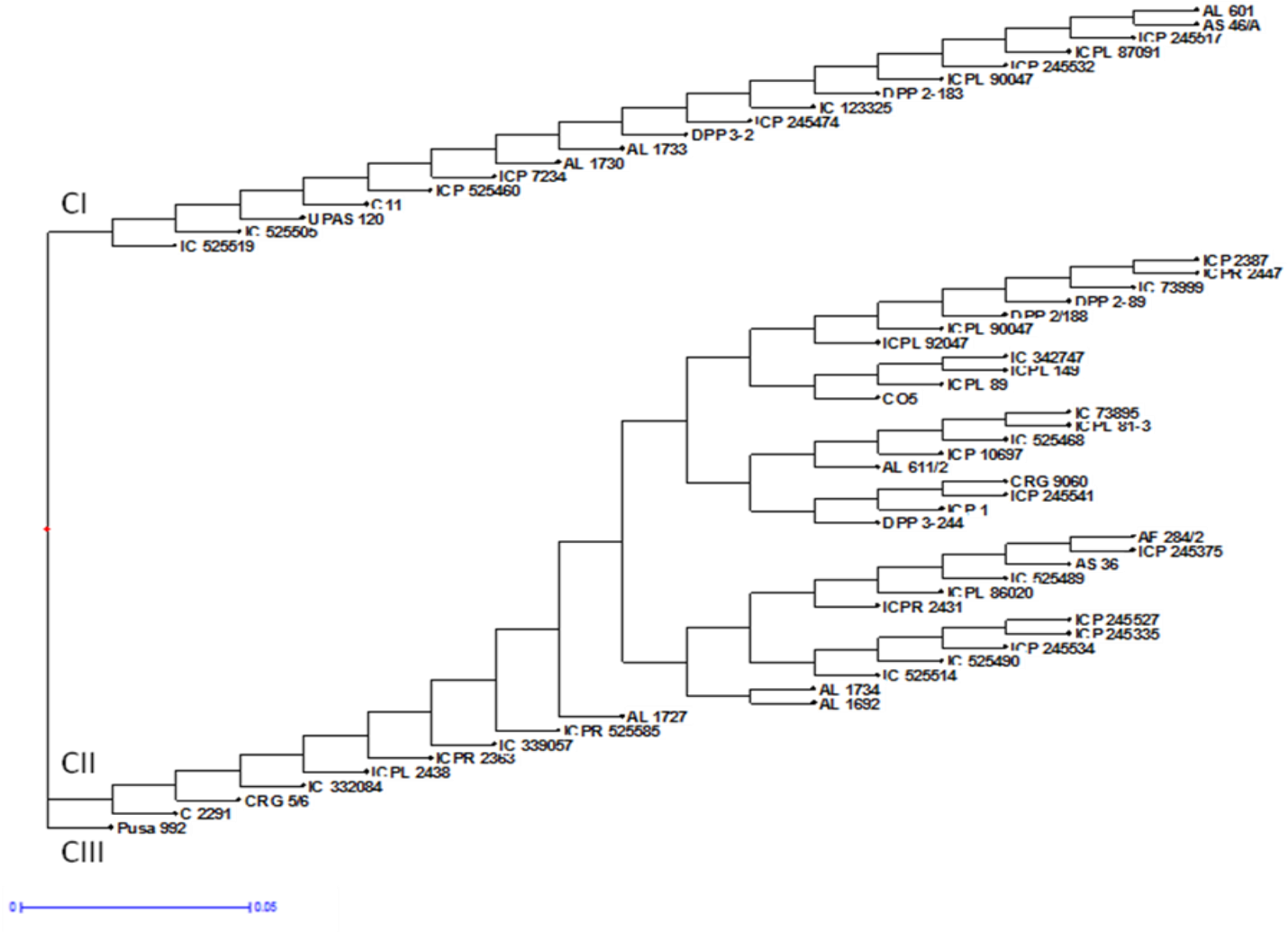

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Fig. 2. Neighbour-joining tree showing the dissimilarity index of the pigeonpea accessions

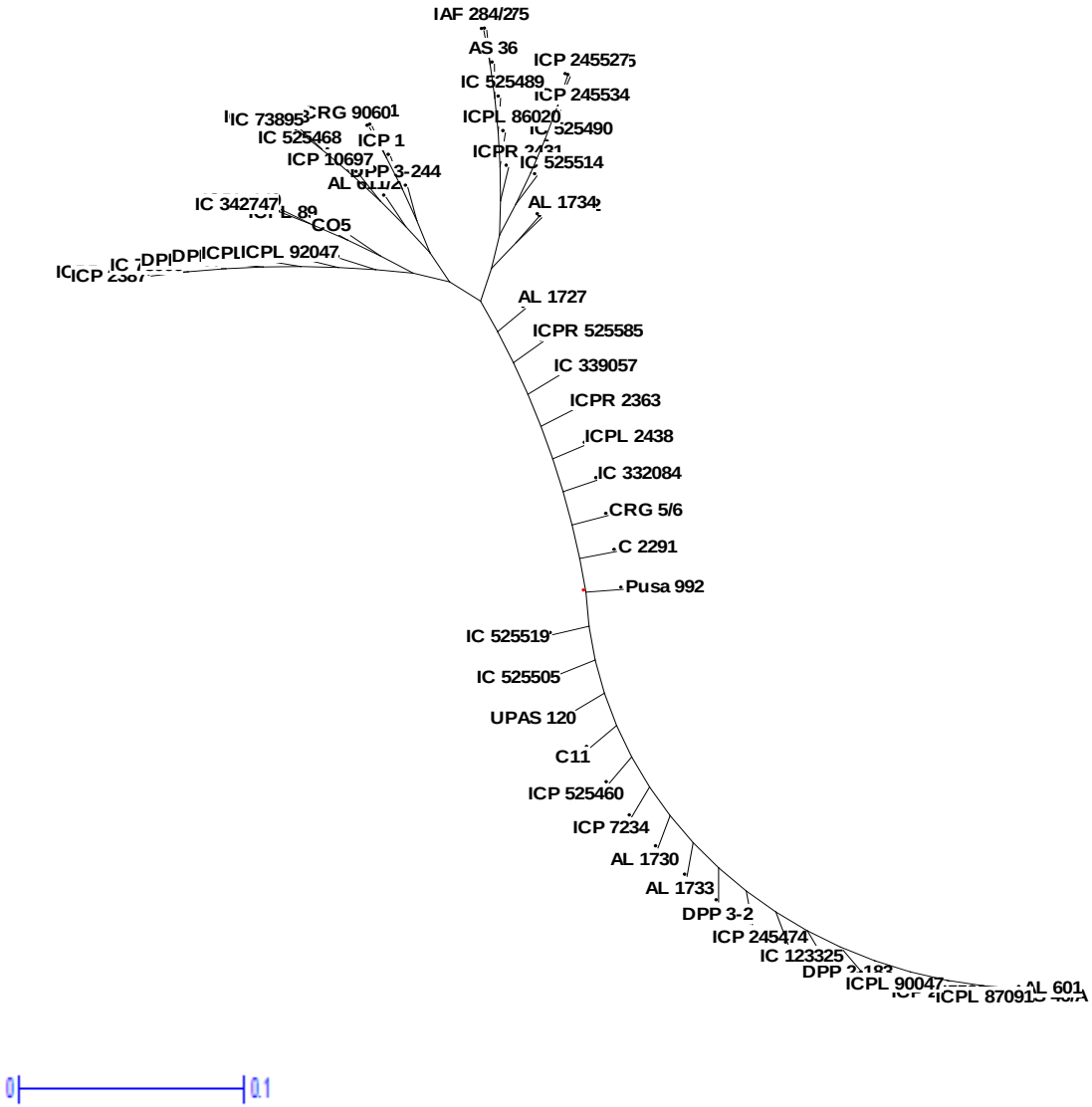

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Fig. 3. SSR polymorphic marker profile of CcM 0399