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MOLECULAR GENETIC DIVERSITY IN GREENGRAM (*Vigna radiata* (L.) Wilczek)

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ABSTRACT

DNA markers were used to characterize the genetic diversity among the germplasm and considered as a promising tool for better understanding of genetic relatedness among the cross derivatives and helps in exploitation of greengram germplasm. The degree of polymorphism exhibited by amplified SSR markers was 50 per cent reflecting the high genetic diversity among the germplasm with 2.47 alleles per marker. The dendrogram based on the Unweighted Pair Group Method using Arithmetic average (UPGMA) hierarchical clustering pattern revealed the 37 genotypes into eight major clusters. All the cultivated genotypes of greengram were grouped together (I), wild progenitor species *Vigna radiata* var. *sublobata*/2 and its derivatives were grouped together (II), whereas all other wild species formed into a separate group (III). The clustering pattern observed in the present study revealed the high level of molecular variability and could be used in the improvement of greengram and determines the genetic relationship among the germplasm.
Keywords : Greengram; SSR markers; molecular genetic diversity; UPGMA.

Introduction

Greengram (*Vigna radiata* (L.) Wilczek), an important diploid legume crop with 2n=2x=22 having genome size of 579 Mbp which belongs to the family Fabaceae (Arumuganathan *et al.*, 1991). It is a short duration and low input requirement crop which can be used in cereal based cropping system. It is rich in vitamin B content and once it is allowed to sprout the ascorbic acid is synthesised fully. It consists of 24 per cent of protein. It can be used as split or whole grains and is considered as one of the easily digestible source of protein diet. It is also used as green manuring crop, which adds nitrogen in addition to humus to the soil. Due to symbiosis with rhizobial bacteria, it plays a major role in nitrogen fixation and used as a cover crop. Due to considerable agronomic value, it is grown in arid and semi arid regions.

In India nearly eight per cent of the area occupied by mungbean, which is the third important pulse crop of India in terms of area cultivated and production next to chickpea and pigeonpea. The area under mungbean in the India is around 5.5 million hectares with a production of 3.1 million tonnes with productivity of 570 kg/ha (Indiastat, 2021-22).

It is a soil protecting crop in rainy season. The productivity of the crop is low due to prevailing biotic and abiotic stresses as well as the lack of assessment of sufficient diversity for yield contributing traits. The development of new mung bean cultivars would increase the yield but was delayed by traditional breeding. Therefore, it is essential to have insight into the genetic diversity present in germplasm for future use and for a subsequent breeding effort (Santalla *et al*., 1998).The germplasm ensures the novel genes and their combination to use after identification (Romero *et al.*, 2001; Kanavi *et al*., 2019). The genetic diversity specifies the uniqueness of the individual genotype in a population or within the species. The genetic diversity play an important role in identification of diverse genotypes which could be utilized in future breeding programme through selection. The genetic diversity based on morphological aspects is laborious, time consuming and affected by many environmental factors (Zeng *et al.*, 2004). Hence, selection based on markers is most effective (Astarini *et al.*, 2004).

The development of molecular marker technology and polymerase chain reaction (PCR) made it simple to characterise crop species molecularly for future genetic advancement (Poczai *et al.*, 2013). The molecular markers *viz.*, ISSR (Smith *et al*., 1997), RAPD (Sathya and Jayamani, 2013; Mwangi *et al.*, 2021), AFLP (Bhat *et al.*, 2005), and SSR markers (Wang *et al.*, 2015) are some of the markers utilised in genetic studies. The RAPD and AFLP are dominant markers and are not reproducible. Microsatellites or SSRs have emerged as extremely attractive markers because of their high repeatability, codominance, polymorphic and multiallelic nature; hence, they are employed in this study (Ramiah, 1953). Due to the several interference of morphological markers, molecular markers which are not affected by environmental factors has significant role in diversity analysis which enhance the efficiency of selection based on genotypes not on phenotypes. The main objective of the study was to characterize the genotypes using SSR markers and to find out the genetic relatedness among the different greengram genotypes and their wild species.

Materials and Methods

The experiment for molecular study was conducted using 37 genotypes (18 germplasm, ten cross derivatives of VBN (Gg) 2 x *Vigna radiata var. sublobata*/2 and nine wild *Vigna* species) of greengram in Marker Aided Selection Laboratory in Department of Pulses, Tamil Nadu Agricultural University, Coimbatore. Molecular analysis was carried out using a set of 22 SSR markers derived from common-bean (*Phaseolus vulgaris*), 33 markers from adzuki-bean (*Vigna angularis*) and five markers from greengram (*Vigna radiata*). For DNA extraction, leaves from 10 days old plant were collected and CTAB method (Doyle and Doyle, 1987) was followed. To avoid RNA contamination the extracted DNA was treated using 5 µl of RNAase. The DNA quality (2 µl DNA and 3 µl orange dye) was checked using 0.8 % agarose gel electrophoresis. The DNA quantity check was done using Nanodrop (Genway Nano, Cole-Parmer, UK) at 260/230 and 260/280nm and 50ng concentration of DNA was used for PCR amplification.

The isolated DNA was subjected to PCR reactions with a volume of 15 µl (7.6 µl of sterile water, 1.5 µl of 10 X buffer, 0.3 µl of 10mM dNTP's, 0.3 µl of 25 mM $MgCl_2$, 3.0 µl of 5 µM primer, 0.3 µl of *Taq* DNA polymerase and 2.0 µl of DNA) containing 50 ng of genomic DNA and amplification was performed in Master cycler gradient PCR (Eppendorf). The PCR condition for SSR marker amplification was follows: Initial denaturation at 94^0 C for 3 minutes, followed by 35 cycles of denaturation at 94^0 C for 45 seconds, annealing temperature at 45 to 60^0 C for 1 minute, extension at 72^0 C for 1 minute and final extension at 72^0 C for 10 minutes. The PCR amplified products were subjected to gel electrophoresis in 3 per cent agarose gel for 3 hours at 100 V (BIO-RAD, USA). The obtained bands were visualized by 1 per cent ethidium Bromide (EtBr) and documented under Ultra Violet light using BIO-RAD gel documentation unit (Gel Doc^{TM} XR^{+}, BIO-RAD, USA). To identify the minimal base pair difference, the amplified PCR products were also subjected to 8 per cent Poly Acrylamide Gel Electrophoresis. The 100 bp DNA ladder (GeneDirex) was used to assess the molecular weight of the amplified DNA product.

The SSR gels were scored and depicted by their allele sizes as allelic data. The dissimilarity coefficient was used to generate a dendrogram for assessing the relationship using UPGMA (Unweighted Pair Group Method with Arithmetic average) (Lakhanpaul *et al.,* 2000) and Neighbour-joining tree generated by the software DARwin 5.0. Polymorphic Information Content (PIC) values were calculated for SSR markers to characterize the capacity of each marker to detect polymorphic loci among the genotypes. PIC value was calculated using the formula $PIC=1-\sum pi^2$, where, *pi* is the frequency of the '*i*'th allele (Smith *et al.,* 1997).

Results and Discussion

In the present study, the SSR markers selected were derived from common-bean (Blair *et al*., 2003) adzuki-bean (Han *et al*., 2005; Chaitieng *et al*., 2006) and greengram (Wang *et al*., 2015; Souframanien and Gopalakrishna, 2009). Among the 60 SSR markers, 40 markers showed amplification and 20 remain unamplified. With the 40 amplified markers, 20 markers exhibited polymorphism, 14 showed monomorphism and 6 produced multiple bands. Among the 22 common-bean markers, nine markers were amplified and the per cent of amplification was 40.91 per cent. With the nine markers, two markers were found to be polymorphic with the polymorphism of 22.22 per cent. With regard to 33 adzuki-bean markers, 26 markers showed amplification with 78.79 per cent of amplification. Among the amplified markers, 15 markers showed polymorphism (57.69 %). In greengram markers, all the five SSR markers were amplified in which three markers showed polymorphism with 60 per cent (Table I). The per cent polymorphism obtained in this study using greengram markers was more when compared to other diverse species of greengram, namely 57.8 per cent (Bangar *et al.,* 2018) and 60 per cent (Tangphatsornruang *et al.,* 2009).

The number of allele expresses the richness of germplasm (Santalla *et al.,* 1998). A total of 84 alleles were generated by 34 markers. The number of alleles produced by above markers ranged from two to five with an average of 2.47 alleles per marker which indicates the high level of genetic diversity. The average number of alleles in this study was more than that of many other species *viz.,* 2.3 alleles in greengram (Gupta *et al.,* 2013), 1.5 alleles in greengram (Kumar *et al.,* 2001), 2.2 alleles in cowpea (Devi and Jayamani, 2020) and 2.87 alleles in mungbean (Ngangkham *et al.,* 2019). The highest number of alleles (5) was recorded by CEDG 097 and CEDG 154 whereas, lowest number of alleles (2) was observed by CEDG 133, CEDG 171 and DMB SSR 014.

Allele size varied from 100 to 300 bp. The allele size in this study was higher when compared to the allele size of 50 bp to 250 bp (Singh, 2013) in greengram. Common-bean derived SSR markers produced alleles ranged from 170 to 290 bp. In adzuki-bean SSR derived markers, allele size ranged from 100 to 280 bp and in greengram derived markers, it varied from 150 to 300 bp.

Polymorphic Information Content (PIC) reveals the amount of information that can be obtained from a particular marker. The markers expressing PIC value of more than 0.5 represents the informativeness of that marker (Jasim *et al.,* 2018). The PIC value was calculated for 20 polymorphic SSR markers. PIC value was highest for the marker DMB SSR 014 (0.905) followed by the marker CEDG 133 (0.846) while, the lowest PIC value recorded by the marker CEDG 149 (0.307). The mean PIC value for 20 polymorphic markers was 0.549 (Table II). The average PIC value was higher in this study than the average PIC value of 0.49 (Gupta and Goplalakrishna, 2009), 0.48 (Srimathy and Jayamani, 2010), 0.39 (Suman *et al.,* 2019) and 0.48 (Gupta *et al.,* 2013) in blackgram. The marker DMB SSR 014 had the highest PIC value of 0.905 followed by the marker CEDG 133 (0.846) which could be used in the field of taxonomical and genetic resource management of *Vigna* crops.

The PIC value related to relative frequency and allele number was directly proportional to polymorphic locus (Jasim *et al.,* 2013). The PIC value of more than 0.5 was observed by a total of thirteen markers which is 65 per cent indicating that these markers could be used for genetic diversity and DNA fingerprinting studies.

Common-bean derived SSR markers BM 14 and BM 170; Adzuki-bean derived markers *viz.,* CEDG 133, CEDG 171, CEDG 010, CEDG 257, CEDG 271, CEDGAAG 001, CEDG 037, CEDG 065, CEDG 085, CEDG 149, CEDG 198,

CEDG 254, CEDG 282, CEDG 097 and CEDG 154; greengram derived markers DMB SSR 014, DMB SSR 020 and DMB SSR 167 showed polymorphism among the cultivated genotypes. They also showed polymorphism among the parents and cross derivatives of VBN (Gg) 2 and *Vigna radiata* var. *sublobata*/2. Hence, these markers could be used to construct linkage map and to map agronomically important genes in greengram.

To have better understanding the relationship, the genotypes were subjected to Dendrogram based on Unweighted Pair Group Method with Arithmetic mean which grouped the 37 genotypes into eight clusters (Fig. 1). Among the eight clusters, cluster I was the largest with 18 genotypes followed by cluster II with 11 genotypes represents the genetic similarity between these genotypes. Cluster II had the entire cross derivatives of VBN (Gg) 2 and *Vigna radiata* var. *sublobata*/2 along with parent *V. radiata* var. *sublobata/2.* Cluster III (TMV (Mb) 1 and *V. aconitifolia*) and VIII (*V. trilobata 2* and *V. trilobata 3*) had two genotypes each whereas other clusters IV (*V. radiata* var *sublobata/1*), cluster V (*V. umbellata*), cluster VI (*V. trilobata 1*) and cluster VII (*V. glabrescence*) occupy solitary cluster each. Similarly, through UPGMA method, 23 genotypes were grouped into three clusters (Kumar *et al*., 2001) in greengram, fifty six genotypes were grouped into eight clusters (Kaur *et al*., 2016) in greengram.

The neighbour-joining tree developed based on weighted average for dissimilarity matrix grouped the 37 genotypes into three groups (Fig. 2). All the cultivated genotypes *viz*., Annur 1, VC 6197 A, SML 668, NM 54, Binamung 7, VC 7960-88, VC 6157-70P, SML 1023, VC 6040A, EC 396117, VC 7890 A, Barimung 5, VC 1997 A, Barimung 7, Co (Gg) 7, VBN (Gg) 3, Co 6 and VBN (Gg) 2 comes under Group I. Cross derivatives of VBN (Gg) 2 and *Vigna radiata* var. *sublobata*/2 along with parent *V. radiata* var. *sublobata/2* comes under Group II. Wild species *viz*., *V. aconitifolia*, TMV (Mb) 1, *V. acontifolia*, *V. radiata* var. *sublobata/1*, *V. trilobata/1*, *V. trilobata/2*, *V. trilobata/3*, *V. umbellata*, *V. glabresence* were distinctly separated from cultivated genotypes and comes under Group III.

In both UPGMA analysis and neighbourhood joining tree, all the cultivated genotypes were grouped together. Cross derivatives along with *Vigna radiata* var. *sublobata/2* also grouped under one cluster and all other wild relatives grouped in other cluster.

Conclusion

From this, it was concluded that the cross derivatives were reflecting the same characteristics of one of the parent *Vigna radiata* var. *sublobata/2*. The SSR markers obtained from the study differentiate the clustering pattern among cultivated and wild species. The polymorphic markers and species specific alleles identified could be further utilized for DNA fingerprinting studies, selective breeding and also in the interspecific hybridization programme in greengram.

Table I : Cross species amplification of SSR markers derived from commonbean, adzukibean and greengram.

S. No	Crop	Number of markers screened	Markers amplified	Markers not amplified	Markers amplified			Per cent of amplification	Per cent of polymorphism
					Monomorphic	Polymorphic	Multiple bands		
1	Commonbean	22	9	13	4	2	3	40.91	22.22
2	Adzukibean	33	26	7	9	15	2	78.79	57.69
3	Greengram	5	5	0	1	3	1	100.00	60.00
	Total	**60**	**40**	**20**	**14**	**20**	**6**	**-**	**-**

Table II. SSR marker analysis using 37 greengram genotypes

S. No.	Marker	Annealing temperature (˚C)	Allele size (bp)	Number of alleles	PIC value	Remarks	Reference
Commonbean							
1	BMarc14	46	210	1	0.000	Monomorphic	Blair *et al.*, 2003
2	BMarc16	55	280	1	0.000	Monomorphic	Blair *et al.*, 2003
3	BM14	50	270-290	3	0.530	Polymorphic	Blair *et al.*, 2003
4	BM170	50	170-200	3	0.734	Polymorphic	Blair *et al.*, 2003
5	BMd 8	50	180	1	0.000	Monomorphic	Blair *et al.*, 2003
6	BMd 26	55	190	1	0.000	Monomorphic	Blair *et al.*, 2003
Adzukibean							
7	CED AAG 001	55	230-260	4	0.520	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
8	CEDG 008	60	130	1	0.000	Monomorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
9	CEDG 010	60	170-190	3	0.610	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006

S. No.	Marker	Annealing temperature (˚C)	Allele size (bp)	Number of alleles	PIC value	Remarks	Reference
10	CEDG 036	55	180	1	0.000	Monomorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
11	CEDG 037	60	120-150	4	0.625	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
12	CEDG 065	60	120-170	4	0.434	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
13	CEDG 085	60	140-180	4	0.503	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
14	CEDG 088	65	210	1	0.000	Monomorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
15	CEDG 097	60	100-130	5	0.651	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
16	CEDG 133	60	110-120	2	0.846	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
17	CEDG 143	60	180	1	0.000	Monomorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
18	CEDG 149	60	170-200	4	0.307	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
19	CEDG 154	60	230-280	5	0.730	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
20	CEDG 156	60	130	1	0.000	Monomorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
21	CEDG 171	60	200-210	2	0.401	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
22	CEDG 176	60	150	1	0.000	Monomorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
23	CEDG 178	55	170	1	0.000	Monomorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
24	CEDG 198	55	200-230	4	0.490	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
25	CEDG 204	55	180	1	0.000	Monomorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
26	CEDG 254	60	150-210	4	0.640	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
27	CEDG 257	55	120-140	3	0.411	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
28	CEDG 271	60	190-240	3	0.466	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
29	CEDG 282	60	140-200	4	0.429	Polymorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
30	CEDG 305	60	120	1	0.000	Monomorphic	Han *et al.*, 2005; Chaitieng *et al.*, 2006
Greengram							
31	DMB SSR 014	55	150-170	2	0.905	Polymorphic	Wang *et al.*, 2015*;* Souframanien *et al.*, 2009
32	DMB SSR 020	56	190-250	3	0.505	Polymorphic	Wang *et al.*, 2015*;* Souframanien *et al.*, 2009
33	DMB SSR 135	55	280	1	0.000	Monomorphic	Wang *et al.*, 2015*;* Souframanien *et al.*, 2009
34	DMB SSR 167	55	250-300	4	0.547	Polymorphic	Wang *et al.*, 2015*;* Souframanien *et al.*, 2009
Total				84	-	-	
Mean				2.47	0.549	-	

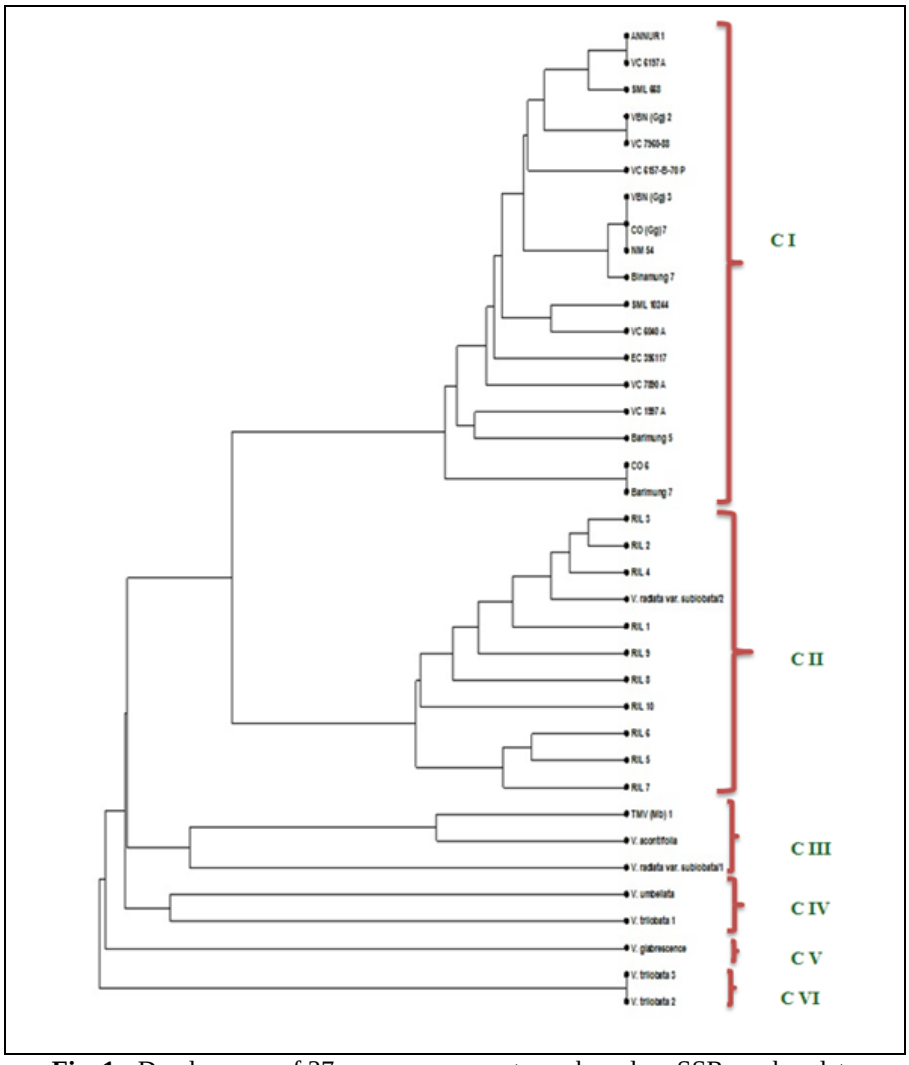

Fig. 1 : Dendrogram of 37 greengram genotypes based on SSR marker data

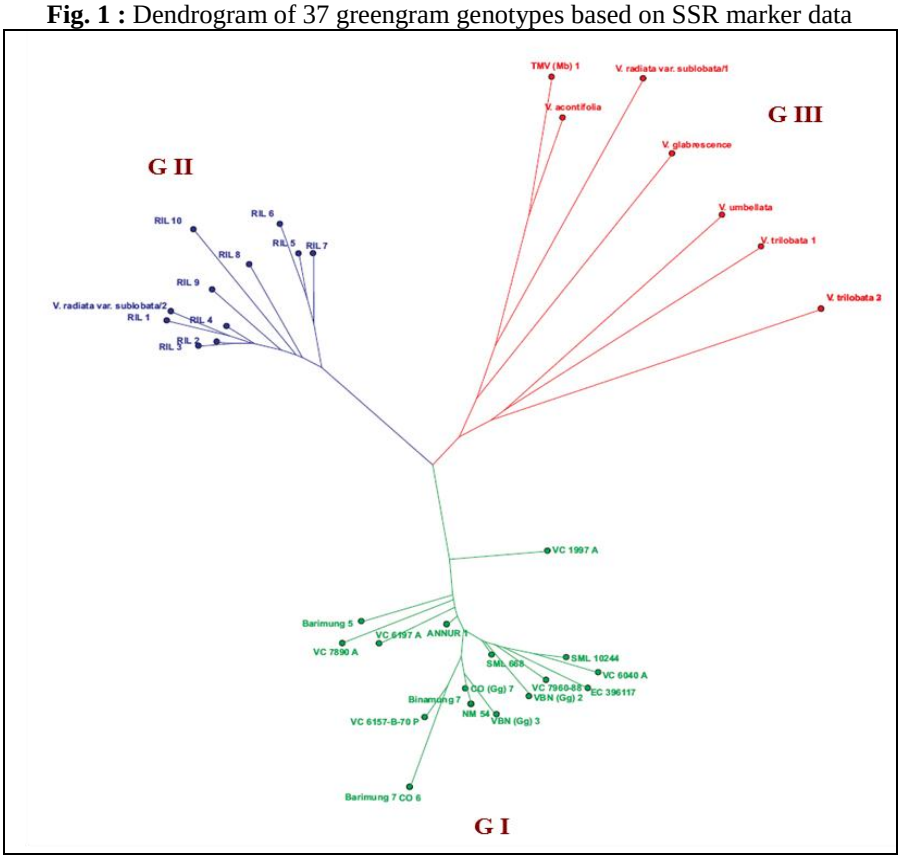

Fig. 2 : Neighbour- joining tree of 37 greengram genotypes based on SSR marker data

Declarations

Conflict of interest: The authors declare no conflict of interest.

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