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Assessment of genetic diversity of soybean genotypes differing in resistance against yellow mosaic virus using simple sequence repeat markers

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ABSTRACT

Yellow mosaic virus disease is one of the major diseases of soybean in India. Genetic basis of 41 soybean genotypes varying in resistance against yellow mosaic virus was studied using 58 simple sequence repeat primers. A total of 140 alleles with an average of 2.41 alleles per locus were detected, which indicated very narrow genetic base of the genotypes studied. The polymorphic information content varied from 0.00 to 0.754 with an average of 0.357. Unweighted Pair Group Method with Arithmetic Average allocated the genotypes in 2 major clusters separated at 41% similarity with fairly good bootstrap support. Ten unique alleles observed in the study can be used for identification of genotype possessing that particular unique allele. The resistant group comprised of 21 genotypes with similarity coefficient 0.33 to 0.805, of which 20 genotypes were classified in a single sub-cluster. The results presented are of significance with regard to the identification of suitable donor parents for incorporating yellow mosaic resistance into popular soybean varieties. Genetically diverse parents with varying resistance against yellow mosaic virus identified in the study can be used for generating mapping population for the identification of SSR markers closely linked with yellow mosaic virus.

Key words: Allele, Soybean, Genetic diversity, Simple sequence repeats, Yellow mosaic virus.

INTRODUCTION

Soybean is the numero uno oilseed crop in the country. According to the fourth estimate of cropping year 2013-14, the production figure for the crop stands at 11.99 million tonnes of the total 32 million tonnes oilseeds produced in the country. In addition, the crop contributes immensely (approximately Rs 7000 crores) to the foreign exchange by the virtue of export of soymeal obtained after extraction of the oil. However, the productivity of the crop which hovers around 1.2 tonne per ha in our country is the major concern compared to the world average of 2.5 tonne per ha. The failure in harnessing the yield potential of released varieties has been ascribed to several biotic and abiotic factors. Of the biotic factors, yellow mosaic virus has been reported to cause significant yield loss in soybean in North India in early 70s (Suteri, 1974) when the magnitude of the loss due to the disease was reported to be as high as 80% (Nene *et al.*, 1972). The disease is caused by the white fly (*Bemisia tabaci*), and is not transmitted by seed, soil or sap. Nucleotide sequence of the virus isolated from soybean plants affected by yellow mosaic disease showed 89% similarity with *Mungbean Yellow Mosaic India Virus* (MYMIV) and was designated as soybean isolate of MYMIV (MYMIV-[Sb]) by Usharani *et al.* (2004). In recent years, the virus has been reported to pose serious threat to the crop in parts of central and South India (Usharani *et al.*, 2004; Raj *et al.*, 2006; The Hindu, 2010). Development of yellow mosaic virus resistant varieties for these specific soybean growing regions remains a challenge for the plant breeders. For this purpose, selection of genetically diverse parents with resistance against yellow mosaic virus is the first prerequisite in plant breeding programme aimed at development of YMV resistant soybean varieties.

Genetic diversity analysis provides the insight for selection of appropriate parents for combining new alleles for the trait in a crop improvement programme. More recently, simple sequence repeats (SSRs) markers, because of their co-dominance, polymorphic and reproducible properties have been employed to measure genetic diversity in soybean (Li *et al.*,2010; Guan *et al.*, 2010; Tantasawat *et al.*, 2011), chickpea (Naghvi *et al*., 2012), wheat (Chen *et al*., 2012), rice (Yadav *et al*., 2013), potato (Carputo *et al*., 2013). Infact, they are DNA sequences that consist of two to five nucleotide core units such as (AT)n, (CTT)n and (ATGT)n. The regions flanking these tandem repeat sequences are conserved in a crop across the genotypes; however the number of repeats may vary in the genotypes thereby resulting in different lengths of PCR amplified products. Even with in very closely related cultivars, the variation in number of repeating nucleotides may occur at

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the same locus. Therefore, SSR markers have been used for the selection of genetically diverse parents for crossing programme for the improvement of particular traits across different crops including soybean (Varshney and Tuberosa, 2010; Singh *et al.*, 2010; Rakshit *et al.*, 2011). Genetic variability for the resistance against yellow mosaic virus in soybean has been reported in the literature (Gadde, 2006). Resistance against a disease in the diverse genetic sources may be due to different genes. Yadav *et al*. (2015) did whole genome sequencing of MYMIV susceptible variety JS335 and resistant genotype UPSM534 (PI171443) to find out the genomic regions associated with resistance gene. They indicated a SNP on chromosome no.18 with a possible association with MYMIV resistance gene. In soybean, genes imparting resistance against yellow mosaic virus disease have also been reported (Singh and Mullick, 1978; Bhattacharya *et al.*, 1999; Talukdar *et al.*, 2013), thereby indicating the possibility of employing marker assisted selection (MAS) approach for development of yellow mosaic resistant varieties. In the present investigation, forty-one genotypes differing for resistance against yellow mosaic virus were subjected to genetic diversity analysis using SSR markers to select diverse parents for initiating breeding programme to develop yellow mosaic virus resistant soybean varieties. Besides, the selection of diverse parents would help in the generation of mapping population for identification of gene responsible for imparting resistance towards the disease.

MATERIALS AND METHODS

Soybean genotypes were screened for reaction against yellow mosaic virus in the field of Punjab Agricultural University, Ludhiana which is the hotspot for YMV. The pedigree and the centre of origin of these genotypes are given in Table 1.

DNA isolation: Genomic DNA was isolated from the finely ground young leaf tissues following cetyl trimethyl ammonium bromide procedure (Doyle and Doyle, 1990). Purification of DNA was done through phenol: chloroform: isoamyl alcohol method. Purified DNA was quantified through spectroscopic method.

Simple sequence repeats (SSR) analysis: A total of 58 SSR primers were randomly chosen for the analysis from twenty linkage groups of soybean genome. These SSR markers were synthesized by Sigma Aldrich India, Bangalore, India. For simple sequence repeat analysis, the purified DNA was subjected to PCR amplification in 10 µl reaction mixture containing 2 µl DNA (25 ng/µl), 1 µl PCR 10x buffer, 1.1 µl $MgCl_2$ (25 mM), 0.1 µl dNTPs (25 mM), 0.4 µl each forward and reverse SSR primers (30 ng/µl), 0.068 µl *Taq* DNA polymerase (3 units/µl) and 4.932 µl distilled water. DNA was denatured at 94˚C for 2 min followed by 30 cycles each consisting of denaturation at 94˚C for 1 min, primer annealing at 50˚C for 2 min, primer elongation at 72˚C for 3 min and final elongation at 72˚C for 10 min in the thermocycler (MJ Research, model PTC100). Amplified products so obtained were resolved on 3% metaphore agarose gel. Allele size was estimated in comparison with 50 bp DNA ladder (Bangalore genei) by running in extreme left lane.

Data Analysis: Computation was facilitated by the PC based programme NTSYS 2.02 (Rohalf, 1998). Presence and absence of an SSR allele was scored as 1 and 0, respectively. The data of all the SSR alleles was imported to NTedit and created into binary data matrix. Similarity coefficients between paired genotypes were computed using Jaccard's similarity formula through SIMQUAL module. Cluster analysis was carried out to construct dendrogram using Unweighted Pair Group Method with Arithmetic Average (UPGMA). To complement the information obtained from the cluster analysis, bootstrap values over 10000 permutations were determined through Unweighted Neighbor Joining (UNJ) using DARwin 5.0 software (Perrier and Jacquemoud-Collet, 2006). The allelic diversity at a locus was measured by polymorphic information content (PIC), which was determined as $PIC_i = 1-\sum^{n}_{j=1} p^2_{ij}$ where *i* denotes the SSR marker while p_{ij} is frequency of j^{th} allele. Any allele appearing in only one genotype was treated as unique allele.

RESULTS AND DISCUSSION

Seventeen soybean genotypes were scored as susceptible while the remaining 21 genotypes resistant and 3 moderately resistant against the yellow mosaic virus as shown in Table 1. All the SL series (SL688, SL744, SL900, SL982, SL799, SL871, SL795, SL958, SL794, SL295, and SL525) soybean genotypes developed from Punjab Agricultural University were yellow mosaic virus resistant genotypes. Besides, PS1241, PK1024, PK1029, PK416, PK1042, MACS22, PK564, PK1092, DS97-12 and UPSM534 were also resistant to yellow mosaic virus reaction. UPSM534 which is a germplasm line and reported to be resistant to yellow mosaic virus has been used as one of the grandparent in development of genotypes like PK416, PK564 which were resistant to yellow mosaic virus reaction. Further, some of the genotypes like PK308, PS1347 and JS97-52 were moderately resistant.

Primer screening: A total of 58 SSR primer pairs, distributed across 20 linkage groups of soybean were used to amplify specific loci from the genomic DNA of each of the 41 soybean genotypes. The amplified products obtained with each of these primers were resolved on 3% metaphore agarose gel and scrutinized for the polymorphism. Fifty one primers detected polymorphism while seven primers (Satt258, Satt143, Satt050, Satt558, Satt459, Satt575 and Satt314) yielded monomorphic bands. A high percentage of polymorphism (87.93%) detected in this study was consistent with the previous studies (Singh *et al.*, 2010; Tantasawat *et al.*, 2011). A total of 140 alleles were amplified with an average of 2.41 alleles per locus, which is slightly higher

Table 1: Soybean genotypes (41) with their respective pedigree and country of origin and the reaction against yellow mosaic virus

Genotype	Pedigree	Reaction	Origin	Genotype	Pedigree	Reaction	Origin
EC537960	EC537960	S	India	PK564	UPSM534xAnkurx Bragg	R	India
JS79-264	JS79-264	S	India	PK1042	Bragg x PK416	R	India
UPSM534	Germplasm accession	R	China	PK1092	PK327 x PK416	R	India
EC39139	EC391349	S	India	JS97-52	PK327 x L129	MR	India
MACS22	—-	R	India	JS79-81	Bragg x Harsoy	S	India
SL688	PK416x SL317	R	India	JS90-41	PS73-7 x Hark	S	India
SL744	SL457xSL459	R	India	JS93-05	Selection from PS73-22	S	India
SL900	PK1241xJS335	R	India	SL295	PK416 x PK564	R	India
SL982	SL525xDS98-41	R	India	SL525	PK416 x PK1023	R	India
SL799	JS90-29x E4	R	India	Samrat	Farmers' selection	S	India
SL871	DS97-12x SL798	R	India	Shivalik	PK7355selection	S	India
SL795	PK1162x E4	R	India	MAUS32	Selection JS8021	S	India
SL958	SL525x SL706	R	India	LSb1	Selection MACS330	S	India
SL794	PK1162xSL459	R	India	NRC7	Selection S69-96	S	India
PS1347	PS1024x PK472	MR	India	ADT1	Selection from "Hill	S	Introduction from USA
PS1241	PK1039xPK327	R	India	Lee	S-100 x CNS	S	Introduction from USA
PK1024	PK308x PK317	R	India	DS97-12	Mutant of DS 74	R	India
PK1029	PK262x PK317	R	India	JS95-60	Selection from PS7322	S	India
PK416	UPSM534xS38	R	India	Hardee	D49-772xImproved pelican	S	Introduction from USA
PK472	Hardee xPunjab1	S	India	JS335	JS78-77x JS 71-05	S	India
PK308	T31xHardee	MR	India				

than as reported by Kumar *et.al.*, (2015). This indicated relatively a very narrow genetic base of the genotypes used in the study. The number of alleles per primer pair (locus) ranged from 1 (Satt258, Satt143, Satt050, Satt558, Satt459, Satt575, Satt314) to 5 (Sct_199, Satt009) as given in Table 2. Three loci amplified 4 alleles, 19 loci showed 3 alleles and 27 loci showed 2 alleles. Representative banding pattern of PCR product profiles at Sct_199 with 5 distinct alleles is shown in Fig 1. Of the total number of 140 alleles, 54 alleles showed a frequency of 0.25 or less, 22 alleles exhibited a frequency of 0.75 or higher and remaining 64 alleles exhibited a frequency between 0.25-0.75. The size of the allele fragments ranged from 80 to 380 bp. The PIC value, which is a measure of allelic diversity, for the 58 SSR markers ranged from 0.00 to 0.754 with 0.357 as the average PIC/ locus. In general, the primer pair showing high number of alleles (4 or 5) also showed high PIC value. Two SSR markers (Sct_199, Satt009) with 5 alleles showed PIC values greater than 0.7. Hence, these 2 SSR markers were the most informative for distinguishing the soybean genotypes. The SSR allelic diversity observed in the present case was moderate compared to the previous reports. Wang *et al.* (2006) with an analysis of 60 SSR markers on 129 soybean genotypes reported an average of 12.20 alleles per locus with average PIC value of 0.78. Similarly, Chotiyarnwong *et al.* (2007) reported an average of 11.83 alleles per locus in an analysis of 149 Thai indigenous and 11 recommended soybean varieties using 18 SSR markers. Low diversity in our study may be because of the fact that fair number of the genotypes selected in the diversity analysis is Indian varieties which have common parentage or one of the parents is variety introduced from the late maturity group of United States.

Of the total 140 alleles identified in the present investigation, 10 alleles (7.14%) were unique which were amplified in single genotype. Satt281, Satt197, Satt190, Satt548, AI856415, Satt181, Satt240, Satt552, Satt571, Satt260 produced one unique allele of fragment size 190, 140, 245, 230, 200, 240, 260, 150, 150, 250 bp respectively. These unique markers indicated by asterisk (*) in Table 2 may be deployed for the efficient identification of some of the genotypes. Some of the yellow mosaic resistant genotypes *viz*. UPSM534, MACS22 and DS97-12 can be identified by the unique alleles of size 150, 240 and 230 bp amplified by Satt552 , Satt181, Satt548, respectively. SL688 can be identified by unique allele (260 bp) amplified by Satt240. Satt598 on linkage group E generated an allele of 190 bp in only three genotypes UPSM534, SL799 and SL958 which were resistant against yellow mosaic virus.

Genetic diversity and relationship among the soybean genotypes: All 140 SSR alleles were used for the genetic diversity analysis. Jaccard's similarity coefficient was calculated to assess the genetic proximity among the genotypes and the similarity coefficient matrix was used for UPGMA cluster analysis. The pair-wise genetic similarity value among soybean genotypes varied from 0.302 to 0.805. SL794 *vs* SL795 was the closest pair (0.805) with the bootstrap value of 98% followed by SL871 vs PK416 (0.753). On the other hand, MAUS32 *vs.* JS90-41 was the most diverse pair (0.3012) followed by MAUS32 *vs*. JS93-05 (0.3023). However, both the genotypes in each of these two combinations of minimum similarity index were sensitive to yellow mosaic virus. Within resistant groups, the most diverse combination was SL958 *vs*. MACS22 followed by SL525 *vs*. MACS22 with similarity values of 0.322 and 0.348, respectively. However, in the pairing of one resistant and one susceptible genotype, SL525 *vs.* LSb1 and MACS22 *vs*. JS93-05 were the two most diverse combinations with similarity index of 0.317 and 0.318, respectively.

Cluster analysis based upon the coefficient of similarity classified 41 soybean genotypes into 2 major groups *viz.* cluster I and cluster II (Fig.2) separating at 41% similarity. Figure 3 depicts the bootstrap value of different pair of genotypes. Cluster I contained just three genotypes *viz*. MACS22, MAUS32, and JS79-264. The cluster II comprised of 2 major subgroups *viz*. IIa and IIb. Subgroup IIa contained 2 yellow mosaic sensitive genotypes *viz.* NRC7 and JS93-05 with 54% genetic similarity. Cluster IIb is the largest subgroup which is further subdivided into IIb1 and IIb2 at 45.5% similarity. In cluster IIb1, of the 11 genotypes, only two genotypes SL794 and SL795, which showed 81%

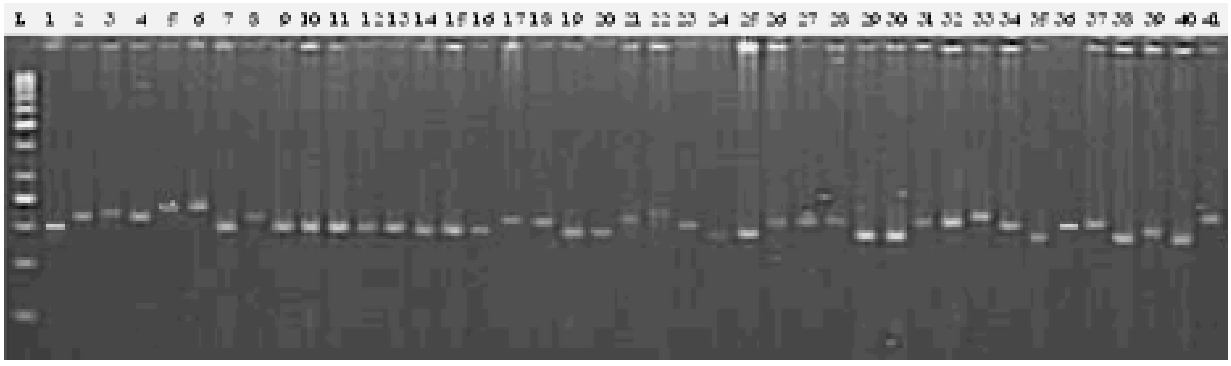

Fig 1: SSR profile of soybean genotypes showing allelic variation at loci Sct_199 (LGp G). L denotes 50 bp ladder.

Table 2: Primer sequence, linkage group, number of alleles and polymorphism information content (PIC) of SSR primers employed in the genetic diversity analysis.

Primer	LGp	Alleles	PIC	Size (bp)	Primer	LGp	Alleles	PIC	Size (bp)
Satt258	A1	1	0.000	240	Satt256	D2	2	0.180	230, 250
Satt200	A1	2	0.477	210, 250	Satt598	E	3	0.461	160, 170, 190
Satt050	A1	1	0.000	200	Sat_124	E	3	0.615	230, 250, 260
Satt228	A2	3	0.421	220, 240, 250	Satt575	E	1	0.000	220
Satt538	A2	3	0.561	110, 115, 125	Satt146	F	3	0.569	300, 320, 380
AW132402	A2	2	0.473	150, 160	Satt114	F	4	0.66	80,100,105, 145
Sat_270	B1	3	0.472	200, 210, 250	Satt586	F	3	0.364	200, 205, 240
Satt509	B1	2	0.277	190, 259	Sct_199	G	5	0.746	190, 200, 210,225, 240
Satt197	B1	2	0.047	140*, 190	Satt612	G	2	0.488	240, 245
Satt577	B2	3	0.565	100, 110, 120	Satt352	G	3	0.528	160, 185, 200
Sat_424	B2	2	0.404	190, 200	Satt181	H	3	0.522	180, 210, 240*
Satt189	B2	2	0.499	160, 195	Satt541	H	3	0.571	160, 170, 180
Sat_140	C1	3	0.272	200, 210, 240	Satt314	H	1	0.000	250
Satt399	C1	3	0.344	290, 300, 340	Satt587	I	2	0.426	160, 170
Satt190	C1	2	0.049	195, 245*	Satt571	I	3	0.433	140,150*,160
GMAC7L	C2	2	0.475	100, 125	AW310961	J	2	0.411	170, 190
Satt305	C2	3	0.468	210, 220, 240	Sat_366	J	2	0.092	190, 210
Satt281	C2	4	0.575	190*,200, 240, 250	Satt285	J	2	0.398	200, 220
Satt457	C2	3	0.236	250, 260, 290	Satt240	K	4	0.624	200,235, 240, 260*
Sat_246	C2	2	0.25	200, 250	Satt552	K	3	0.144	135, 150*,155
Satt643	C2	2	0.32	250, 260	Satt260	K	2	0.047	230, 250*
Satt658	C2	2	0.176	235, 240	Satt229	L	2	0.525	200, 225
Satt267	D1a	3	0.536	220, 230, 240	Satt143	L	1	0.000	260
Satt502	D1a	2	0.567	250, 255	Satt523	L	2	0.288	170, 190
Satt548	D1a	3	0.255	230*, 245, 250	Satt551	M	2	0.482	230, 240
Satt558	D1b	1	0.000	240	Satt009	N	5	0.754	160,170, 180, 210, 240
AI856415	D1b	2	0.048	200*, 205	Sat_132	O	2	0.493	250, 255
Sat459	D1b	1	0.000	180	Sat_318	O	2	0.315	150, 200
Satt002	D2	2	0.398	140, 145	Satt345	O	2	0.184	240, 250

***** Allele amplified in the genomic DNA of single genotype.

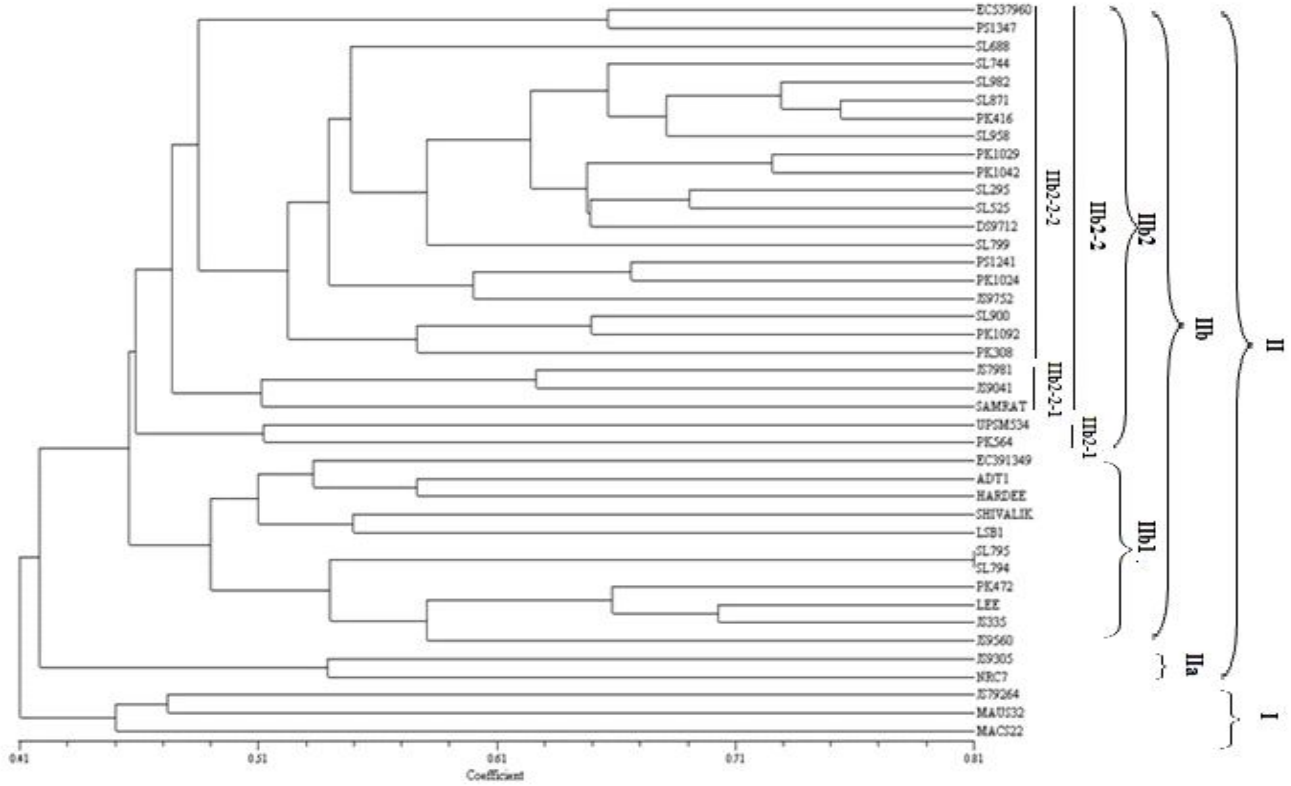

Fig 2: UPGMA dendrogram showing similarity coefficients and genetic relationships among 41 soybean genotypes based on SSR profile.

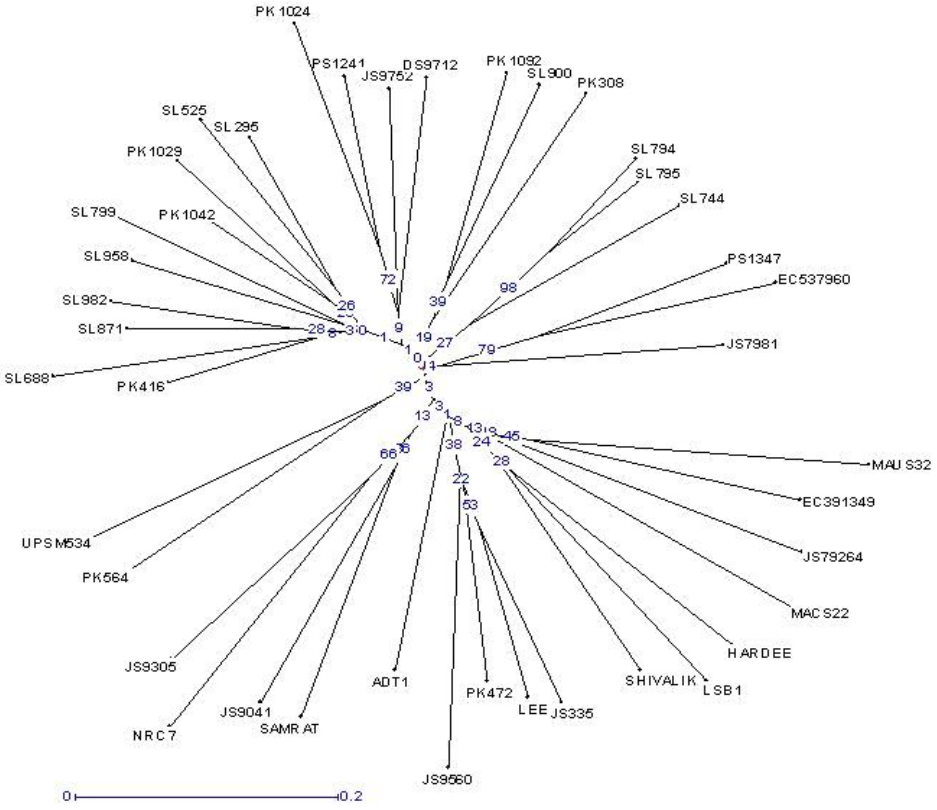

Fig 3: Unweighted Neighbor-Joining dendrogram prepared using DARwin showing clustering pattern of 41 soybean genotypes based on SSR data. Values at nodes are percentage over 10,000 bootstrap replicates.

similarity to each other with bootstrap value of 98%, were resistant against yellow mosaic virus while the reaction of the remaining 9 genotypes was sensitive. IIb2 is further sub-divided into subgroups IIb2-1 and IIb2-2 with 46% similarity. In subgroup IIb2-1, the reaction of both the genotypes PK564 and UPSM534 against yellow mosaic virus was resistant and the genotypes showed 52 % similarity. Infact, UPSM534 is the known source for the yellow mosaic virus resistance while PK564 has been derived from UPSM534. The subgroup IIb2-2 is divided into IIb2-2-1 and IIb2-2-2 at 47 % similarity with bootstrap value of 39%. IIb2-2-1 contained three YMV sensitive genotypes (Samrat, JS90-41 and JS79-81). IIb2-2-2 is the largest sub-cluster with 20 genotypes, all which showed distinct/moderate resistance against yellow mosaic virus except EC537960 which was susceptible to yellow mosaic virus. Mantel's test for cophenetic correlation with r = 0.882 indicated a good fit of the soybean genotypes in a group in the cluster analysis. Genotypes JS335, JS93-05, NRC7 and JS95-60 are the elite Indian soybean cultivars but all are sensitive to yellow mosaic virus. Based upon the similarity index, from the resistant genotypes observed in the study, both JS95-60 and JS335 were found to be the most diverse from PK1029 with similarity coefficient of 0.342 and 0.386, respectively. JS93-05 and NRC7 were found to be the most diverse from MACS22 and SL688 with similarity coefficient of 0.318 and 0.341, respectively. These parental combinations are suggested to develop high yielding cultivars with resistance against yellow mosaic virus. Parents with diverse genetic background within resistant group are desirable in the plant breeding programme for pyramiding of diverse resistant genes, which is less prone to resistance breakdown. Our results showed that the most diverse parental combinations observed in SL958 *vs*. MACS22 followed by SL525 *vs*. MACS22 would be the most appropriate to generate mapping population for tagging genes for resistance against yellow mosaic virus.

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