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Molecular Characterization and Genetic Diversity Assessment of Soybean Varieties using SSR Markers

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A B S T R A C T

Soybean (*Glycine max* (L.) Merrill] one of nature's most versatile crops is increasingly becoming an important food and cash crop in the tropics due to its high nutrient quality and adaptability to various growing environments. Soybean is a grain legume crop. As food and feed soybean plays an important role throughout the different countries of the world. It provides oil as well as protein to the living beings. In present study Molecular characterization and genetic diversity assessment of soybean varieties was done using SSR markers. For this eight Soybean varieties were selected and 54 SSRs primer pairs, distributed across the integrated linkage map of soybean were used. The 8 varieties of soybean were profiled with 54 polymorphic SSR markers which produced 216 alleles. The allele number for each SSR locus varied from two to six with an average of 4.00. The fragment size of these 216 alleles was ranged from 95 to 437 bp. The number of alleles per primer pair (locus) ranged from 2 (Satt 207, Satt 671, Satt 414 and Satt 327) to 6 for Satt 552, Sat_107, Satt 002 and Satt 323 with an average of 4.00. All loci were polymorphic and were detected by Gene Tool software version 4.03.05.0. In the clustering pattern the dendogram generated based on SSR markers grouped the 08 Soybean varieties into two clusters having 06 and 02 varieties respectively.

Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the world's most important economic legume crops. A number of cultivars have been released in India from different soybean breeding centres for growing under different agro climatic conditions by introduction, selection, mutation and hybridization of elite cultivars and germplasm through systemic breeding and evaluation programmes (Chauhan *et al.*, 2015). Generations of new and improved cultivars can be enhanced by new sources of genetic variation; therefore criteria for parental stock selection need to be considered not only by agronomic value, but also for genetic dissimilarity. Therefore, understanding the genetic diversity of soybean germplasm is essential to broaden the genetic base and to further utilize it in breeding program (Kumawat *et al.*, 2015). Knowledge on genetic diversity in soybean

could help to understand the structure of germplasm, predict which combinations would produce the best offspring and facilitate to widen the genetic basis of breeding material for selection.

With the introduction of PPV & FRA 2001, the need for precise genotype characterization for varietal identification and clear distinctness has attained a greater importance. Such an insight could be achieved through molecular characterization of soybean germplasm using DNA markers, which are more informative, stable and reliable, as compared to morphological and molecular markers. Among different types of DNA markers being utilized for molecular characterization and genetic diversity analysis in plants, simple sequence repeats (SSR) markers are considered as molecular marker of choice due to their abundance, high polymorphism rate and high reproducibility. SSR markers have been widely used in the genetic diversity studies of the soybean germplasm collections worldwide and high levels of polymorphism at SSR loci have been reported for both the number of alleles per locus and the gene diversity (Maughan *et al.,* 1995; Abe *et al.,* 2003; Wang *et al.,* 2006, 2010; Fu *et al.,* 2007; Wang and Takahata 2007; Li *et al.,*2008; Singh *et al.,* 2010; Tantasawat *et al.,* 2011). Early studies have shown utilization of molecular markers for identification of genetically diverse genotypes to use in crosses in breeding programme (Maughan *et al.,* 1996; Thompson and Nelson 1998).

Keeping the above view, the present investigation was carried out with an objective to study the diversity level among the genotypes and to identification of specific marker for particular genotype. Genetic distances will further help in identifying genetically diverse genotypes, which then can be utilized in creating valuable selectable variation.

Materials and Methods

Plant materials

The plant material comprises of eight soybean varieties in active seed multiplication chain developed and released by JNKVV, Jabalpur (Table 1). The seeds were obtained from the Seed Breeding Farm, Department of Plant Breeding & Genetics, JNKVV, Jabalpur (MP).

DNA Extraction

Total genomic DNA was isolated from fresh young leaves following the CTAB (cetyl trimethyl ammonium bromide) procedure as described by Saghai Maroof *et al.,* (1984) with some modifications. Quantification of DNA was accomplished by analyzing the DNA on 0.8% agarose gel stained with ethidium bromide using diluted uncut lambda DNA as standard. Final concentration was adjusted to 50ngµl^{-1} for further uses in PCR analysis.

PCR amplification

A total of 54 SSRs primer pairs, distributed across the integrated linkage map of soybean (Cregan *et al.,* 1999) were used. The details of SSR markers, their sequences and motifs are given in table 2. DNA was amplified by PCR using our previously standardized method (Sahu *et al.,* 2012) in a total volume of 10 µl containing 2X PCR assay buffer, 1.5mM $MgCl_2$, 100µM of each dNTPs, 12ng each of forward and reverse primers, 0.2 units of Taq DNA polymerase and 25 ng of genomic DNA template. Amplification reaction initiated with a 5-minute pre-denaturation steps at 94^0 C followed by 35 cycles of DNA denaturation at 94^0 C for 30 seconds, primer annealing at 50-55^0 C for 30 seconds and DNA extension at 72^0 C for 7 minutes was performed after 35 cycles. Amplified PCR products was

separated on 2.0% of agarose gel at a volage of 90V for the period of 45 minutes to 1 hour in 1X TBE buffer stained with ethidium bromide. The gel was visualized in UV transilluminator and photograph taken using Syngen make gel documentation system.

SSR allele scoring and data analysis

The presence or absence of SSR fragment in each accession was recorded for all the polymorphic SSR markers. The SSR bands appearing without ambiguity were scored as 1 (present) and 0 (absent) for each primer. The size of the amplified product was calculated on the basis of its mobility relative to molecular mass of marker (100 bp DNA ladder). The genetic similarity among genotypes was estimated based on Jaccard's similarity coefficient. The resulting similarity matrix was further analysed using the unweighted pair-group method arithmetic average (UPGMA) clustering algorithm for construction of dendrogram; the computations were carried out using NTSYSpc version 2.2 (Rohlf 2000).

Results and Discussion

SSR polymorphism

Molecular characterization of germplasm accessions reveals underlying allelic diversity and genetic base of germplasm collection. In the present study a total of 54 SSR primer pairs, distributed on different linkage groups of soybean (Cregan *et al.,* 1999), were used. The 8 varieties of soybean were profiled with 54 polymorphic SSR markers which produced 216 alleles. The allele number for each SSR locus varied from two to six with an average of 4.00. The fragment size of these 216 alleles was ranged from 95 to 437 bp. The high percentage of polymorphic SSR loci detected in this study was consistent with previous studies (Maughan *et al.,* 1995; Rongwen *et al.,* 1995; Diwan and Cregan 1997; Narveletal. 2000; Kumar *et al.,* 2009; Singh *et al.,* 2010; Bisen *et al.,* 2015). The number of alleles per primer pair (locus) ranged from 2 (Satt 207, Satt 671, Satt 414 and Satt 327) to 6 for Satt 552, Sat_107, Satt 002 and Satt 323 with an average of 4.00 (Table 3 and Fig. 1).

Identification of unique allele

Presence of unique band helped in the identification of specific genotype and may be useful for DNA fingerprinting. Such markers are highly reliable in the establishment of genetic relatedness among the genotypes. Similar results were reported by Jain *et al.,* (1994), Srivastava *et al.,* (2001), and Vinu *et al.,* (2013) in different crop species. Different unique alleles were amplified by eighteen different SSR loci viz., Satt 215 for JS 97-52, Satt 519 for JS 20-29, Satt 244 and Satt 364 for JS 20-69, Satt 152, Sat_167, Satt 598 and Satt 154 for JS 20-34, Satt 453, Satt 294 and Satt 446 for JS 93-05, Satt 523 for JS 95-60, Satt 369, Satt 386, Satt 267 and Satt 337 for JS 20-98 and Satt 146, Satt 552 for JS 335 (Table 3). The genotypes identified for these unique alleles can be used in marker assisted introgression program but further validation is required for marker traits linkage in segregating populations.

Genetic relationship among soybean varieties

Cluster analysis was used to group the varieties and to construct a dendogram. The dendogram generated based on SSR markers grouped the 08 soybean varieties in two clusters. Cluster I comprised of two sub-clusters. Sub-cluster I comprised of four varieties i.e. JS 93-05, JS 20-69, JS 20-29 and JS 97-52. Sub-cluster II comprised of two soybean varieties i.e. JS 95-60 and JS 20-34.cluster II comprised of two soybean varieties i.e. JS 20-98 and JS 335 (Fig. 1 and 2).

Table.1 SSR markers with their sequences selected for the study (http://www.soybase.org)

Primers	Reverse sequence	Forward sequence	Amplification temperature (^{o}C)
Satt 146	GTG GTG GTG GTG AAA ACT ATT AGA A	AAG GGA TCC CTC AAC TGA CTG	55
Sat_268	GCG TGA GGA GGT TCA AAA ATA ACA T	GCG TGC AAC ATA TGA CAC CAT AAA T	55
Satt 270	GCG CAG TGC ATG GTT TTC TCA	TGT GAT GCC CCT TTT CT	55
Satt 207	GCG ATT GTG ATT GTA GTC CCT AAA	GCG TTT TTC TCA TTT TGA TTC CTA AAC	55
Satt 369	GCG AGT TCG AAT TTC TTT TCA AGT	AAC ATC CAA AGA AAT GTG TTC ACA A	55
Satt 309	GCG CCT TAA ATA AAA CCC GAA ACT	GCG CCT TCA AAT TGG CGT CTT	55
Sat_243	GCG GCA ACC GCT TAA AAA TAA TTT AAG AT	GCG ATG TCG AAT GAT TAT TAA TCA AAA TC	55
Satt 152	TAG GGT TGT CAC TGT TTT GTT CTT A	GCG CTA TTC CTA TCA CAA CAC A	55
Sat_167	TTG AGC CGA AAG TTC AAT TCT A	AAG GCA CTC TTC CAT CAA TAC AA	52
Satt 529	GCA CAA TGA CAA TCA CAT ACA	GCG CAT TAA GGC ATA AAA AAG GAT A	52
Satt 441	AAA TGC ACC CAT CAA TCA CA	AAA CCC ACC CTC AAA AAT AAA AA	52
Satt 598	CAC AAT ACC TGT GGC TGT TAT ACT AT	CGA TTT GAA TAT ACT TAC CGT CTA TA	52
Satt 453	TAG TGG GGA AGG GAA GTT ACC	GCG GAA AAA AAA CAA TAA ACA ACA	52
Satt 318	GCG ATA TTT ATA TGG CCG CTA AG	GCG CAC GTT GAT TTT TTT ATA GTA A	52
Satt 671	GCG AGA AAT GAG ATA AGT GGT GAT A	GCG TAA ATC CAA AAG TAG AAT AAA ATA A	52
Satt 386	CTT CGT TGA TAC CTC AGT AGA GTA CAA A	GCG GAT GAT TTT TAT AGA ATA GAT AAT	52
Satt 281	TGC ATG GCA CGA GAA AGA AGT A	AAG CTC CAC ATG CAG TTC AAA AC	55
Satt 215	CCC ATT CAA TTG AGA TCC AAA ATT AC	GCG CCT TCT TCT GCT AAA TCA	55
Satt 244	GCG ATG GGG ATA TTT TCT TTA TTA TCA G	GCG CCC CAT ATG TTT AAA TTA TAT GGA G	55
Satt 431	GCG CAC GAA AGT TTT TCT GTA ACA	GCG TGG CAC CCT TGA TAA ATA A	55
Satt 519	CCG CAA GGT TAC GAA CTG CTC GAA	GGA TTT CAA AGA ATG AAC ACA GA	55
Satt 523	GCG CTT TTT CGG CTG TTA TTT TTA ACT	GCG ATT TCT TCC TTG AAG AAT TTT CTG	55
Satt 353	GCG AAT GGG AAT GCC TTC TTA TTC TA	CAT ACA CGC ATT GCC TTT CCT GAA	55
Satt 414	GCG TCA TAA TAA TGC CTA GAA CAT AAA	GCG TAT TCC TAG TCA CAT GCT ATT TCA	55
Sat_124	GGG AGT TCA AAC ATC CAT TAG TGG TAT A	GGG TCC ATT CCA CTT TTT GTA CAA TAT	55
Satt 552	GAT CCG CAT TGG TTT CTT ACT T	CGA ACC GGC AAA ACC AAG AT	55
Satt 294	GCG CTC AGT GTG AAA GTT GTT TCT AT	GCG GGT CAA ATG CAA ATT ATT TTT	55
Satt 285	GCG GAC TAA TTC TAT TTT ACA CCA ACA AC	GCG ACA TAT TGC ATT AAA AAC ATA CTT	55
Satt 538	GGG GCG ATA AAC TAG AAC AGG A	GCA GGC TTA TCT TAA GAC AAG T	55
Satt 156	CCA ACT AAT CCC AGG GAC TTA CTT	CGC ACC CCT CAT CCT ATG TA	55
Sat_107	GGA GGA ATT ATT TGG GTT GTA C	TTT GGA AGT ATA AAA TTA TGA ATG ACT	50
Satt 045	ATG CCT CTC CCT CCT	TGG TTT CTA CTT TCT ATA ATT ATT T	50
Satt 160	CAT CAA AAG TTT ATA ACG TGT AGA T	TCC CAC ACA GTT TTC ATA TAA TAT A	50

Satt 267	CAC GGC GTA TTT TTA TTT TG	CCG GTC TGA CCT ATT CTC AT	50
Satt 423	GTT GGG GAA TTA AAA AAA TG	TTC GCT TGG GTT CAG TTA CTT	50
Satt 154	AAA GAA ACG GAA CTA ATA CTA CAT T	AGA TAC TAA CAA GAG GCA TAA AAC T	50
Satt 371	GAG ATC CCG AAA TTT TAG TGT AAC A	TGC AAA CTA ACT GGA TTC ACT CA	50
Satt 002	TCA TTT TGA ATC GTT GAA	TGT GGG TAA AAT AGA TAA AAA T	50
Satt 229	GCG AGG TGG TCT AAA ATT ATT ACC TAT	TGG CAG CAC ACC TGC TAA GGG AAT AAA	58
Satt 557	GCG CAC TAA CCC TTT ATT GAA	GCG GGA TCC ACC ATG TAA TAT GTG	58
Satt 367	GCG GAA TAG TTG CCA AAC AAT AAT C	GCG GAT ATG CCA CTT CTC TCG TGA C	58
Satt 232	GCG GAC ATA AAT GCA ATC ACT TAA AAA G	GCG GCG TGA ATA GTA TAC GTT GAG A	58
Sat_366	GCG GAC ATG GTA CAT CTA TAT TAC GAG TAT T	GCG GCA CAA GAA CAG AGG AAA CTA TT	58
Satt 597	CGA GGC ACA ACC ATC ACC AC	GCT GCA GCG TGT CTG TAG TAT	58
Satt 549	GCG CGC AAC AAT CAC TAG TAC G	GCG GCA AAA CTT TGG AGT ATT GCA A	58
Satt 589	GCG AAA AAG TAA TAT AAG TAG AAA AAG G	GCG CAG ACA ATT TCA GTG GCA GAT AGA	58
Satt 323	TGT GCG TTT AAA TTG CAG CTA AAT	GCG GTC GTC CTA TCT AAT GAA GAG	55
Satt 333	GCG CAA CGA CAT TTT CAC GAA GTT	GCG AAT GGT TTT TGC TGG AAA GTA	55
Satt 327	GCG TCG TAG CAA TGT CAC CA	GCG CAC CCA AAA GAT AAC AAA	55
Satt 337	GCG TAA TAC GCA AAA CAT AAT TAG CCT A	GCG TAA ATC TGA TAT ATG TTA CCA CTG A	55
Satt 364	ATC GGG TCA TGA CTT TTG AAG A	GCG GCA TAA GTT TTC ATC CCA TC	55
Satt 380	GCG TGC CCT TAC TCT CAA AAA AAA A	GCG AGT AAC GGT CTT CTA ACA AGG AAA G	55
Satt 446	GCG GGC AAA TTT GAC CTA ACT CAC AAC	CCG CAT AAA AAA CAC AAC AAA TTA	55
Satt 313	GCG CGA GGT ATG GAA CCT AAC TCA CA	GCG GTA AGT CAT GGC TTT TTA ATC TT	55

Table.2 Number, polymorphic and unique alleles and allele size in soybean involving SSR markers

S no.	Primers	Number of alleles	Polymorphic alleles	Unique alleles	Allele size range (bp)
1	Satt 146	5	5	1	392-437
2	Sat_268	5	5	-	306-354
3	Satt 270	5	5	-	382-426
4	Satt 207	2	2	-	420-426
5	Satt 369	4	4	1	330-355
6	Satt 309	3	3	-	229-239
7	Sat_243	2	2	-	372-381
8	Satt 152	3	3	1	300-330
9	Sat_167	4	4	1	289-305
10	Satt 529	5	5	-	283-311
11	Satt 441	4	4	-	311-340
12	Satt 598	3	3	1	229-243
13	Satt 453	4	4	1	217-234
14	Satt 318	3	3	-	246-259
15	Satt 671	2	2	-	194-200
16	Satt 386	3	3	1	178-189
17	Satt 281	4	4	-	233-251
18	Satt 215	4	4	1	121-133
19	Satt 244	3	3	1	160-200
20	Satt 431	4	4	-	182-200
21	Satt 519	4	4	1	217-234
22	Satt 523	4	4	1	167-183
23	Satt 353	4	4	-	162-183
24	Satt 414	2	2	-	278-285
25	Sat_124	5	5	-	200-218
26	Satt 552	6	6	1	151-180
27	Satt 294	4	4	1	200-269
28	Satt 285	4	4	-	152-169
29	Satt 538	5	5	-	95-120
30	Satt 156	4	4	-	406-433
31	Sat_107	6	6	-	126-204
32	Satt 045	5	5	-	125-148
33	Satt 160	4	4	-	229-243
34	Satt 267	4	4	1	220-318
35	Satt 423	2	2	-	227-246
36	Satt 154	4	4	1	262-326
37	Satt 371	5	5	-	241-272
38	Satt 002	6	6	-	114-137

39	Satt 229	5	5	-	166-214
40	Satt 557	5	5	-	162-195
41	Satt 367	4	4	-	205-219
42	Satt 232	4	4	-	234-256
43	Sat_366	4	4	-	178-194
44	Satt 597	4	4	-	133-150
45	Satt 549	4	4	-	209-229
46	Satt 589	4	4	-	146-168
47	Satt 323	6	6	-	136-159
48	Satt 333	4	4	-	159-179
49	Satt 327	2	2	-	218-224
50	Satt 337	3	3	1	174-184
51	Satt 364	4	4	1	212-231
52	Satt 380	4	4	-	126-139
53	Satt 446	4	4	1	271-300
54	Satt 313	5	5	-	184-218

Table.3 Details of five unique SSR alleles identified

S. No.		Primer	Unique allele Size (bp)	Genotype showing unique allele
1		Satt 215	133	JS 97-52
2		Satt 519	217	JS 20-29
3		Satt 244	200	JS 20-69
4		Satt 364	225	
5		Satt 152	330	JS 20-34
6		Sat_167	305	
7		Satt 598	238	
8		Satt 154	326	
9		Satt 453	217	JS 93-05
10		Satt 294	269	
11		Satt 446	300	
12		Satt 523	167	JS 95-60
13		Satt 369	338	JS 20-98
14		Satt 386	178	
15		Satt 267	318	
16		Satt 337	184	
17		Satt 146	392	JS 335
18		Satt 552	180	

Fig.1 SSR Profiling of Soybean varieties using different SSR markers (M: 100 bp marker, 1: JS 97-52, 2: JS 20-29, 3: JS 20-69, 4: JS 20-34, 5: JS 93-05, 6: JS 95-60, 7: JS 20-98, 8: JS 335)

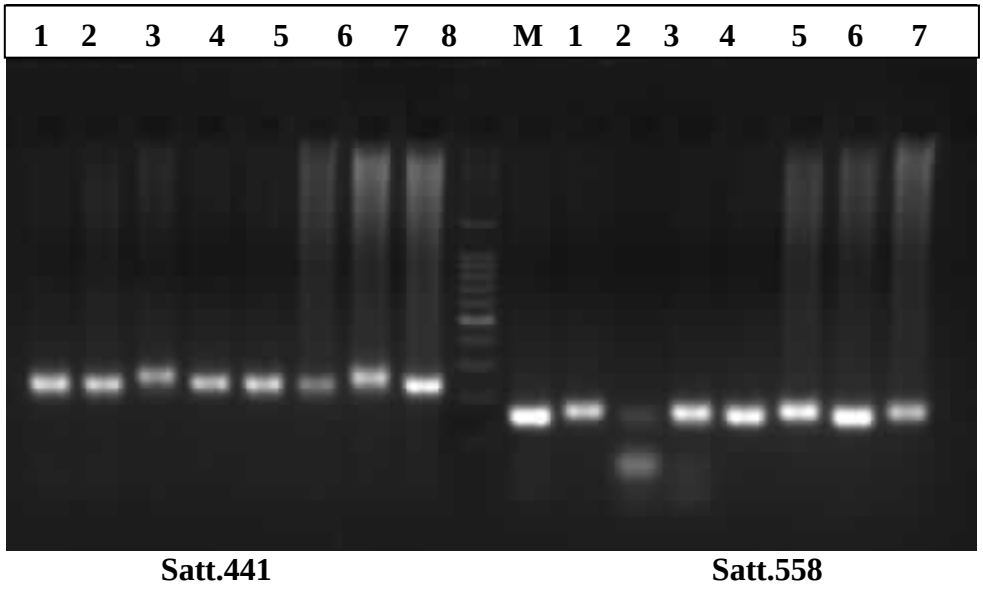


Fig.2 Rooted Dendogram of soybean varieties based on SSR markers

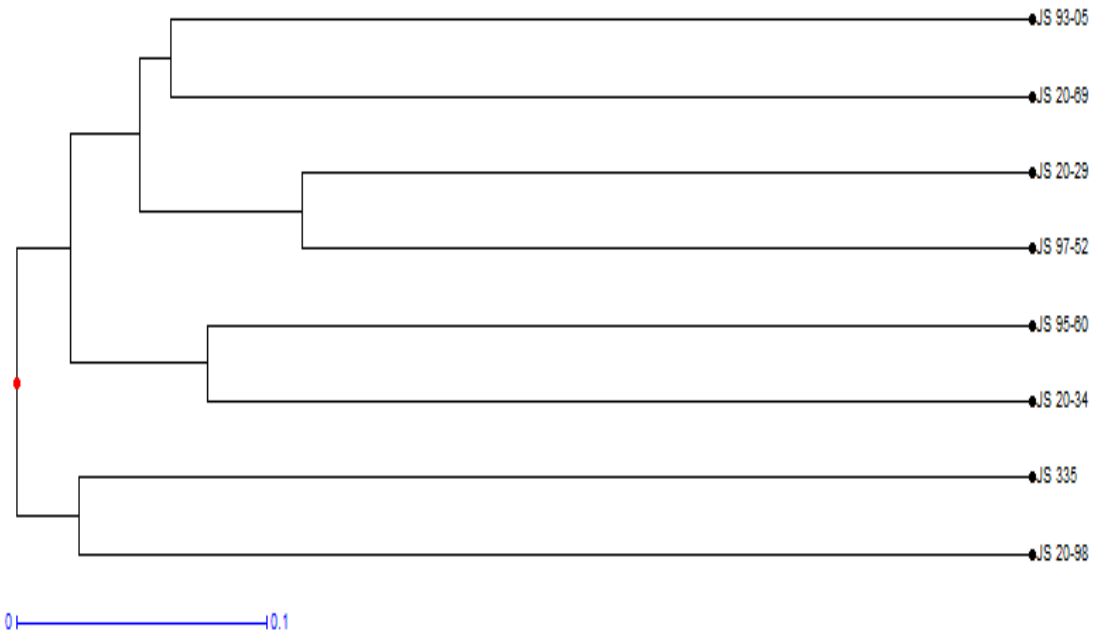
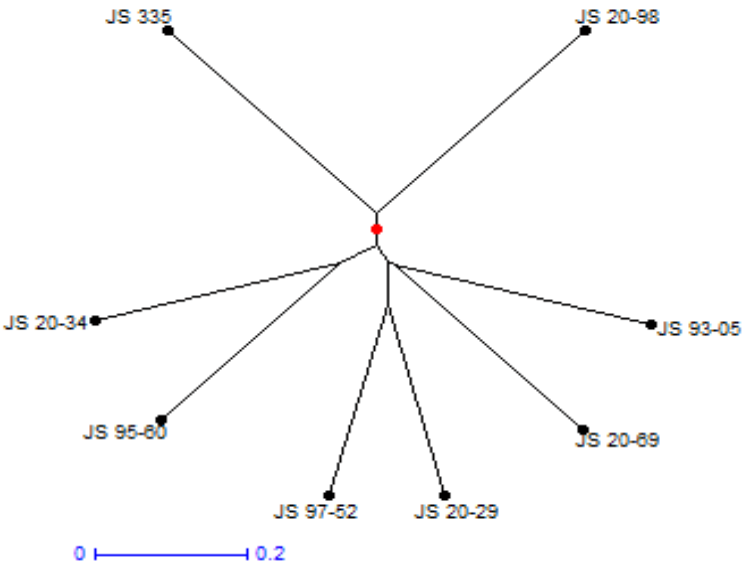


Fig.3 Unrooted Dendrogram of soybean varieties based on SSR markers



Evaluation of genetic divergence and relatedness among breeding materials has significant implications for the improvement of crop plants. Knowledge on genetic diversity in soybean could help breeders and geneticists to understand the structure of germplasm, predict which combinations would produce the best offspring and facilitate to widen the genetic basis of breeding material for selection. Information on genetic distances based on microsatellite markers shall be preferred in creating selectable genetic variation using genotypes which are genetically apart (Vieira *et al.,* 2007; Vinu *et al.,* 2013). The diversity analysis can further be utilized for the development of diverse gene pool. The hybridization among the diverse gene pool will result into more heterotic combinations.

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