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Molecular Characterization and Genetic Diversity Analysis of Commercial Soybean (Glycine Max L. Merr.) Genotypes with SSR Marker

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Abstract

Soybean [*Glycine max* (L.) Merrill] is the world's most economically important legume crop it provides high-quality nutrients with the richest amount of protein. The current study is based on molecular markers, Simple Sequence Repeats (SSRs) to determine the genetic diversity of genotypes of soybeans. Two varieties viz., AMS 155 and CSB 09-03 exhibited a score of nine (9) in both years thus providing the highly susceptible varieties. Among the polymorphic SSR markers, the highest genetic diversity was observed with Satt256 (0.6948) while the lowest genetic diversity was observed with Satt299 (0.4179). Similarly, polymorphism information content (PIC) was highest (0.8274) in Satt114 and lowest (0.3729) in Satt070 among all polymorphic markers used for the identification of genetic diversity. These results advise that the uses of SSR markers are very efficient for measuring genetic diversity and relatedness as well as identifying soybean varieties. Genetic materials (DNA & RNA) may be used for the improvement of soybean genotypes genetically to increase productivity in the country.

Keywords: Begomovirus, Soybean, Genetic Diversity, SSR Marker, PCR, Molecular Marker

Introduction

Soybean (Glycine max L.) is one of the economically important legume crops in the world. It has many nutritional properties for human and livestock feeding. Soybean belongs to the

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, 2022

Leguminosae family which provides proteins and oil for the human diet (Dragoljobet. al, 1999). Soybean is a native of China and an important legume crop cultivated worldwide as a vegetable oil source (Olufemiet. al, 2010; Agarwal et. al,2013). Soybean is a nutrient supplier as a high-valuable protein that is a good source of amino acids (Lubungu et. al., 2013; Singh et. al.,1987; Weingartner, 1987). In just a few decades, India began commercial soybean farming, and since then, both the cultivated area and overall production have grown remarkably. (Tiwari et. al.,1999). Given the current state of oilseed production in India, soybeans are the most widely planted crop, covering 10.69 million hectares and producing 14.66 million tonnes in total in 2012. Comparing India's soybean productivity to other major soybean-producing nations, it is much lower, coming in at 1370 kg/ha in 2012. It is believed that the genetic bases of soybean cultivars are incredibly limited. (Hymowitz, 1970). Soybean being a self-pollinated crop with limited outcrossing is a highly inbreeding crop. In the past few decades, hybridization programs utilizing a small number of carefully chosen genotypes as parental lines have been the majority of the focus in Indian soybean breeding, resulting in a limited genetic base. To grow soybeans, 108 enhanced varieties have been created and made available in India thus far. On the other hand, the available important genetic diversity of soybean is up till currently being fully utilized to enhance the production of soybean and to become wider the genetic base. Hence, understanding the genetic diversity of soybeans is essential to make a wider genetic base and to be further utilized in breeding programs. Such insight could be achieved through the molecular more informative characterization of soybean germplasm by using DNA markers that are more stable and also reliable, as compared to the predictable methods means pedigree analysis and diversity analysis based on morphologically. Early studies have revealed the utilization of molecular markers for the identification of genotypes which genetically diverse to use in crosses in breeding (Thompson and Nelson, 1998 Maughan, et. al., 1996).

Among the all-various types of markers available for genetic diversity analysis in plants, molecular markers are more capable, specific, and also reliable in discriminating which is closely related to species and cultivars. Simple sequence repeats known as Microsatellites are sequences of short tandem repeats 3 distributed over the genomes. It is one of the important markers for the analysis of polymorphism to detect genetic diversity. In particular, SSRs have been used successfully in the identification of genetic diversity and relationships with the genotypes of soybean in different populations (Wang *et. al.*, 2010; Guan *et. al.*, 2010). Simple sequence repeat markers are also known as microsatellites, these are very useful, reliable, and easy to use for the characterization of soybeans. SSR markers have become very important for the

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, 2022

genetic mapping and genetic diversity which is determined in soybeans because they are codominant and spread right through the genome, exhibit high levels of polymorphism, undergo PCR easily, and can be finally analyzed by using gel electrophoresis (Ramesh *et. al.*, 2016).

The current study in this review was carried out to the understand diversity level between the different genotypes of soybeans. A particular genotype was identified by using markers in another area of the present study. Genetic diversity (distances) will further help in the identification of genetically different genotypes, It can be utilized to create variations that are selectable and valuable.

Therefore, these problems could be overcome by molecular characterization. The present study was undertaken towards the Analysis of genetic diversity based on molecular detection by using an SSR marker for polymorphism analysis.

2. Material and Methods

This study collected 22 genotypes of soybeans from different sources in the Vindhya Region, Madhya Pradesh, India. Areas of sample collection such as the College of Agriculture, Rewa (M.P.), A.K.S. University, Satna (M.P.), Local farmer, Satna (M.P.) (Table.1) (Figure.1). This study used morphologically treated soybean genotypes for the genetic diversity analysis.

Figure 1 Healthy leaves of soybean

Table 1: **Verity of soybean germplasm and source of collection**

S. No.	Verity	Source of Collection
1.	JS20-35	College of Agriculture, Rewa (M.P.).
2.	KDS699	College of Agriculture, Rewa (M.P.).
3.	MAUS504	College of Agriculture, Rewa (M.P.).
4.	RKS66	College of Agriculture, Rewa (M.P.).
5.	NRC89	College of Agriculture, Rewa (M.P.).
6.	DS9814	College of Agriculture, Rewa (M.P.).
7.	VLS81	College of Agriculture, Rewa (M.P.).
8.	AMS56	College of Agriculture, Rewa (M.P.).
9.	JS20-38	College of Agriculture, Rewa (M.P.).

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, 2022

10.	PS1503	A.K.S. University, Satna (M.P.).
11.	MAUS609	A.K.S. University, Satna (M.P.).
12.	AMS155	A.K.S. University, Satna (M.P.).
13.	KSO184	A.K.S. University, Satna (M.P.).
14.	CSB09-03	A.K.S. University, Satna (M.P.).
15.	ASB18	A.K.S. University, Satna (M.P.).
16.	KS112	A.K.S. University, Satna (M.P.).
17	KDS378	A.K.S. University, Satna (M.P.).
18.	RKS68	Local Farmer, Satna (M.P.).
19	KSO184	Local Farmer, Satna (M.P.).
20.	DS9712	Local Farmer, Satna (M.P.).
21.	MAUS611	Local Farmer, Satna (M.P.).
22.	AMS155	Local Farmer, Satna (M.P.).

2.1 Isolation of Genomic DNA

The total genomic DNA was extracted from bulk young leaves of 22 samples of healthy soybean genotypes using the following modified Cetyl trimethyl ammonium bromide method (CTAB) (Saghai et. al., 1984; Doyle and Doyle 1987). Extracted DNA stored at -20ºC for further use.

2.2 Quality Checking of DNA

DNA was checked for its purity and intactness by agarose gel electrophoresis. A part of the DNA sample was run on 0.8 % agarose stained by using Ethidium Bromide following a standard protocol (Sambrook et. al., 1989) to estimate its quality. After gel electrophoresis DNA bands were visualized using a gel documentation system (US Major Science).

2.3 Quantification of DNA

Purity and quantification of the leaf-extracted DNA were done using a spectrophotometer (Systronics, Double beam spectrophotometer 2203). 10 µl of stock DNA was diluted with 1990 µl of sterile double distilled water and O.D. was taken at wavelengths 260 nm and 280 nm. The ratio OD 260 / 280 was determined to measure the purity of the sample. The concentration of the template DNA in the test sample was calculated by using the following formula (Eq 1): $\boldsymbol{DNAConcentration}$ $(\boldsymbol{pg/pl})$ = $\boldsymbol{OD260}$ $(\boldsymbol{nm})$ $\boldsymbol{x}$ $(\boldsymbol{Dilutionfactor})$ $\boldsymbol{x50x10"3}$

..... (1)

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, 2022

2.4 Dilution of DNA

Based on the quantification data, part of each DNA sample was diluted with DEPC (diethylpyrocarbonate) treated in sterilized distilled water to yield a working concentration of 10 ng/µl. The diluted samples were stored in a freeze at 4° C for immediate use, while the original undiluted DNA samples were kept for a long time stored at -20° C.

2.5 Simple Sequence Repeat Markers

A set of 10 SSR markers were used for genetic diversity analysis. The markers were selected covering the whole genome of the Soybean. The sequences of the markers were downloaded from the 'soybase' (http://www.soybase.org) and synthesized and procured through vendors (Sigmaaldrich.com). The list of markers with base sequences and linkage groups is given in table no.2.

Table 2: Selected primer sequence and their linkage group

S. No.	Marker	Linkage group	Primer Sequence
1.	Satt256	A1	F:GCGCGACCTTAATGATA R:GCGCCCAAAGCTTAAAATTTAATA
2.	Satt656	F	F:GCGTACTAAAAATGGCAATTATTTGTTG R:GCGTGTTTCAGTATTTGGATAATAGAAT
3.	Sattl77	A2	F: AGTTTCATTCCCATGCCAATA R:CCCGCATCTTTTTCAACCAC
4.	Satt299	I	F: GCGACAAGGCACTCACATCTCTTCTC R:GCGCTACCCATAACAAAAAGTTCAAATC
5.	Satt460	C2	F:GCGCGATGGGCTGTTGGTTTTTAT R:GCGCATACGATTTGGCATTTTTCTATTG
6.	Satt038	G	F:GGGAATCTTTTTTTCTTTCTATTAAGTT R:GGGCATTGAAATGGTTTTAGTCA
7.	Satt396	C1	F:GCGAAAAGGGATAAGTTTAAAAAT R:GCGGGCCTGTAAAGGGATTCC
8.	Satt114	F	F:GGGTTATCCTCCCCAATA R:ATATGGGATGATAAGGTGAAA
9.	Satt070	B2	F:TAAAAATTAAAATACTAGAAGACAAC R:TGGCATTAGAAAATGATATG
10.	Sattl78	K	F:GGGAAAATTCTTTTCATATAGATG R:GGGGTTGAGATATTTTGTTCATAC

Table 3 Polymerase chain reaction mixture

Reagents	Manufacturer	Concentration	Quantity (µl)
Taq buffer A (with MgCl2)	Biolit, SRL	10X	2.0
NTFS	Do	10 mM	2.0

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, 2022

SSR primer (Forward)	Do	20 ng / µl	2.0
SSR primer (Reverse)	Do	20 ng/ µl	2.0
Taq DNA Polymerase(3U/ µl)	Do	3 U / µl	0.3
Double distilled H2O	Do	6.7	
Template DNA	Do	20 ng/ µl	5.0
Total		**20.0**	

2.6 Polymerase Chain Reaction (PCR) Analysis using SSR

A set of 10 SSR primers were selected covering the whole genome of the Soybean. The genomic DNA isolated from twenty-two soybean genotypes was amplified by using these 10 SSR markers. PCR amplification of the DNA was done in a specific reaction volume of 20 µl. The reaction volume contained all the necessary components in the right concentrations Table No. 3.

The amplification of the DNA sample was performed in a Thermocycler (Sure cycler 8800, Agilent Technology). Thermal profiling was set up with an initial denaturation step temperature of 95 degrees Celsius for 5 min followed by the 35 cycles then denaturation (95 degrees Celcius for 60 seconds), annealing step (45-55°C for 60 seconds), and extension (72 °C for 1min). The step of a final extension was completed at 72° C for 7 minutes.

2.7 Agarose Gel Electrophoresis

On metaphor agarose (3%) gels containing EtBr (0.5 µg per ml) in 1X concentration known as the working solution of TAE buffer, in this process the amplified SSR fragments were separated according to size. The electrophoresis process was carried out at 80-100 V for 1.5 hours. The assembly was switched off once the dye reached the 2/3rd length of the gel. The gel was exposed to a UV transilluminator (US Major Science) for visualizing the bands and photographs were taken using the Gel Documentation System (US Major Science), Allele sizes were analyzed in comparison by using a DNA ladder (100 bp, Fermentas) and subsequently used for scoring leading to the polymorphic study.

2.8 Scoring of SSR Allele and Data Analysis

SSR alleles were scored by using a manual scoring system. Resolved unmistakable polymorphic bands were scored to visualize

their presence or absence. The scores were obtained in the form of a matrix with one (1) and zero (0), which indicates the presence of bands and absence of bands in each genotype, respectively. The genotypes showing bands of two alleles with the same intensity were

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, 2022

considered heterozygous for that locus. Polymorphism information contents (PIC) were calculated based on a formula (Eq 2)

PIC ¼ 1−ΣPi2 ... (2)

Where Pi is the frequency of the first allele in the set of genotypes is analyzed and calculated for each SSR locus. The hereditary relationship of the accessions and variation matrixes of Rogers W were clustered and also calculated by using the system with NTSYSpc software, version 2.2 employing the unweighted pair group method of the arithmetic average, (UPGMA) to create the dendrograms. The Principal Component Analysis (PCA) was performed to support the pattern of genetic diversity providing a graphical representation of diversity among the genotypes in the form of a 2-D image.

3. Results and Observations

DNA isolated from new emerging leaves was successfully done by using the CTAB method. The presence of DNA for the diversity analysis in the sample was tested through electrophoresis in 0.8 % Agarose gel. Clear and thick bands in the gel indicated the presence of template DNA in isolated samples. The quantity of DNA was determined by taking Optical density 1 of samples at A260 / A280 nm in a spectrophotometer means 50µg. The quantity of DNA in all the 22 samples varied from 700-1400 ng /pi which was more than enough for amplification through PCR.

3.1 Polymorphism Information Content (PIC)

PIC was calculated to determine the level of polymorphism among Simple Sequence Repeat (SSR) primers. Our findings from polymorphism analysis revealed genetic variability. The presence of DNA in the sample was tested through electrophoresis. The quantity of DNA was determined by spectrophotometer at 260 nm same study was done by Kachare, et. al., 2019. The quantity of DNA in all 22 samples was amplified through the PCR. In this study, all 10 SSR markers were amplified and produced clear and scorable bands. Out of the 10 SSR markers, five markers were found to have a maximum value of PIC.

3.2 Scoring of SSR Allele and Data Analysis

The number of alleles concerning each SSR marker along with the PIC value is presented in Table No. 6. The number of alleles ranged from two to seven with an average of 3.4 alleles per locus. The highest allelic variability was observed in Satt114 with 7 alleles and Satt256 with 4 alleles. Satt656, Satt177, Satt299, Satt460, Satt038, Satt396, and Sattl78 were the least variable locus each with three (3) alleles whereas Satt070 with 2 alleles. The SSR marker profiles of twenty-two soybean genotypes generated by the primer Satt256, Satt299, Satt460,

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, 2022

Satt114, and Satt070 are presented in Figures No. 2, 3, 4, 5, and 6 respectively. The higher the PIC value, the more informative is the SSR marker. The PIC values of markers ranged from 0.3729 to 0.8274 with an average of 0.5426. Satt114 was found to be highly informative with the highest PIC (0.8274) followed by Satt256 (0.6948). The lowest PIC was found in Satt070 (0.3729) followed by Satt299 (0.4179). According to earlier research (Diwan and Cregan 1997; Narvel et al., 2000; Singh et al., 2010; Tantasawat et al., 2011), this study was trustworthy. The chosen collection of SSR markers, which were previously examined for polymorphism among a range of genotypes, may be responsible for the high rate of SSR polymorphism. [23]. The current set of soybean accessions, however, appears to have limited allelic diversity based on the reduced allele number and PIC values. In comparison to most other research, the SSR allelic diversity found across the genotypes of soybeans in this study was minimal. (Diwan and Cregan 1997; Narvel et. al., 2000; Abe et. al., 2003; Wang et. al., 2006; Guan et. al., 2010; Tantasawat et. al., 2011).

Table 4 Number of allele and PIC of 10 polymorphic SSR markers

S.No.	Marker	No. of Allele	Heterozygosity	PIC
1.	Satt256	4	0.7424	0.6948
2.	Satt656	3	0.5434	0.5197
3.	Satt177	3	0.5924	0.5684
4.	Satt299	3	0.5178	0.4179
5.	Satt460	3	0.5978	0.5258
6.	Satt038	3	0.6524	0.5919
7.	Satt396	3	0.5187	0.4835
8.	Satt114	7	0.8467	0.8274
9.	Satt070	2	0.4959	0.3729
10.	Sattl78	3	0.5189	0.4246

Average of Allele per locus = 3.4,
Average PIC= 0.5426

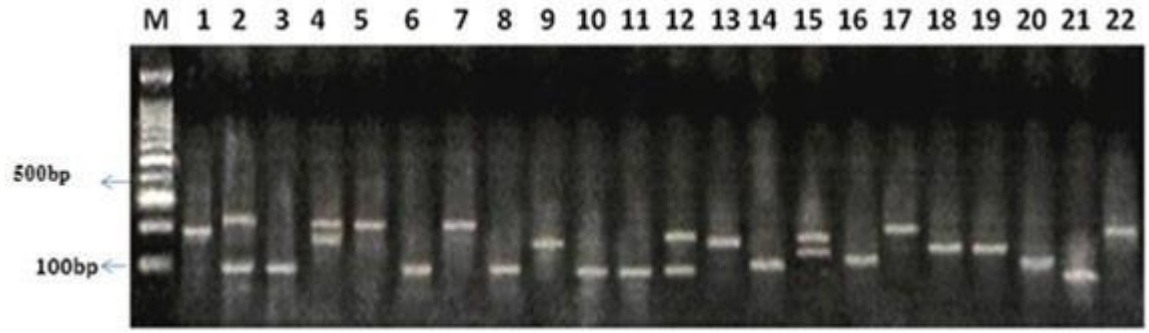

Figure 2PCR amplification with Satt256 primer

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, 2022

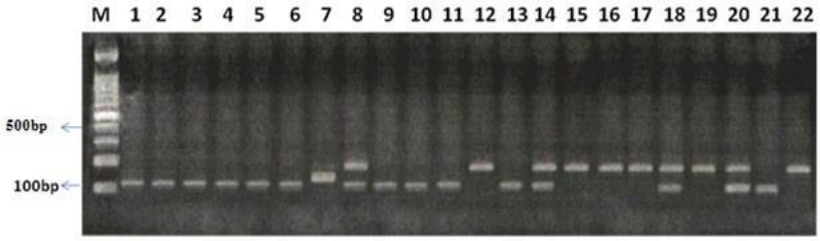

Figure 3 PCR amplification with Satt299 primer

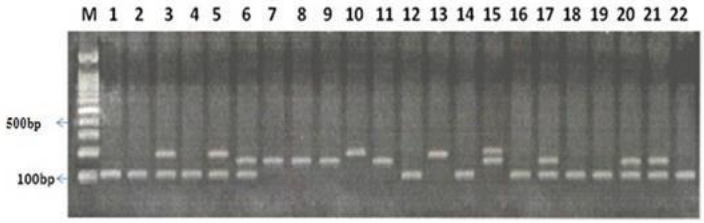

Figure 4 PCR amplification with Satt460 primer

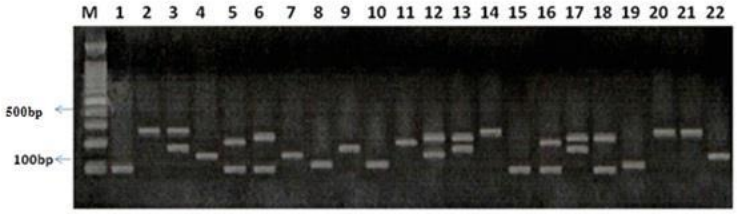

Figure 5 PCR amplification with Satt 114 primer.

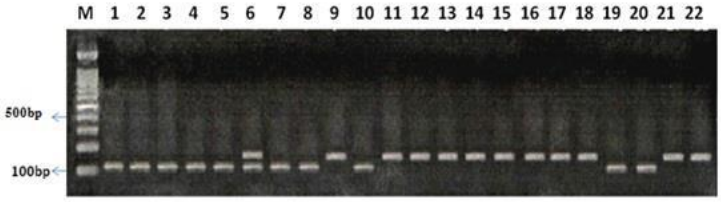

Figure 6 PCR amplification with Satt 070 primer

3.3 Molecular Variability Analysis

This result is by using the UPGMA method to construct the tree. It was developed for constructing the taxonomic phenograms, i.e. trees that reflect the genotypic character similarities between Operational Taxonomic Units (OTUs), but it can also be used for the construction of the phylogenetic trees if the evolution rates are approximately constant with the different lineages. For this purpose determine both the number of observed nucleotides and amino-acid substitutions that can be used. UPGMA employs a sequential clustering algorithm. This research identified the pair with the maximum similarity, and so on until we are left with only 2 (two) OTUs.

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, 2022

Similarity coefficients for the 22 soybeans accessions based on 10 SSR markers. The pair-wise association among 22 soybean accessions showed the highest Distance value of 0.750 and the lowest Distance value of 0.143. The Cophenetic Correlation among 22 soybean verities is 0.736388. The highest polymorphism was observed in primer Satt114. The dendrogram showing the genetic relationships among 22 soybean accessions based on SSR markers is presented in the NCSS dendrogram report. The dendrogram showed three major clusters. Cluster A (primary cluster) consisted of one wild soybean VLS81. Cluster B consisted of 21 varieties of soybean that are polyembryonic in SSR marker. Cluster B is further grouped into two clusters C and D. Cluster C contains DS 9712, ASB 18, KDS 699, RKS66, MAUS611, KDS 378, KSO 184, and JS 20-38and cluster D contains the rest of the varieties.

3.4 Molecular Diversity Analysis

The genetic diversity of the germplasm helps in the selection of the parental genotypes for the development of segregated populations and to development of varieties. Morphological traits-based genetic diversity is level to environmental variations and the availability of a restricted number of morphological markers has limited their use in the studies on genetic diversity. On the other hand, molecular markers-based genetic diversity is not affected by environmental factors hence highly reproducible and also broadly distributed throughout the genome. To well broaden the genetic base of the modern soybean cultivars, an insight into molecular diversity is essential. In this study, an attempt has been made to study the molecular diversity of 22 accession numbers using 10 polymorphic SSR markers. The highest polymorphism observed in the SSR markers along with the soybean accessions in the present study demonstrated the effectiveness of SSR markers for determining genetic variations. The results of the current study revealed that the diversity between the soybean genotypes tested strength assist breeders in the future in the selection of parents for the breeding of soybeans (Kujane et. al., 2019; Marconato et. al., 2016).

Table 5Polymorphic locus in soybean varieties

S.No.	Variety	Satt256	Satt656	Satt177	Satt299	Satt460	Satt038	Satt396	Satt114	Satt070	Satt178
SV1	JS 20-35	0	1	0	0	0	1	1	0	0	0
SV2	KDS 699	1	0	0	0	0	0	0	0	0	0
SV3	MAUS 504	0	0	0	0	1	1	1	1	0	0
SV4	RKS66	1	0	1	0	0	0	0	0	0	1
SV5	NRC89	0	0	0	0	1	0	1	1	0	0
SV6	DS9814	0	1	0	0	1	1	0	1	1	0
SV7	VLS81	0	0	0	0	0	0	0	0	0	0
SV8	AMS 56	0	0	1	1	0	0	1	0	0	0
SV9	JS 20-38	0	0	0	0	0	1	0	0	0	1
SV10	PS 1503	0	1	0	0	0	0	0	0	0	0
SV11	MAUS609	0	1	1	0	0	1	0	0	0	0
SV12	AMS 155	1	0	0	0	0	1	1	1	0	0
SV13	KSO 184	0	0	0	0	0	0	0	1	0	1
SV14	CSB 09-03	0	1	0	1	0	0	0	0	0	0

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, 2022

SV15	ASB 18	1	0	0	0	1	1	0	0	0	0
SV16	KS 112	0	1	0	0	0	0	1	1	0	0
SV17	KDS 378	0	0	1	0	1	0	0	1	0	1
SV18	RKS68	0	1	0	1	0	1	0	1	0	0
SV19	KSO 184	0	0	0	0	0	0	1	0	0	0
SV20	DS 9712	0	0	1	1	1	0	0	0	0	1
SV21	MAUS611	0	0	0	0	1	0	0	0	0	0
SV22	AMS 155	0	1	0	0	0	1	0	0	0	0

1= polymorphic, 0= single allele

Table 6 Similarity index among all soybean varieties

	SV1	SV 2	SV 3	SV 4	SV 5	SV 6	SV 7	SV 8	SV 9	SV10	SV 11	SV 12	SV 13	SV 14	SV 15	SV 16	SV 17	SV 18	SV 19	SV 20	SV 21	SV 22
SV1	1	0.000	0.400	0.000	0.200	0.333	0.000	0.200	0.250	0.333	0.500	0.400	0.000	0.250	0.200	0.500	0.000	0.400	0.333	0.000	0.000	0.667
SV2		1	0.000	0.333	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.333	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SV3			1	0.000	0.750	0.500	0.000	0.167	0.200	0.000	0.167	0.600	0.200	0.000	0.400	0.400	0.333	0.333	0.250	0.143	0.250	0.200
SV4				1	0.000	0.000	0.000	0.200	0.250	0.000	0.200	0.167	0.250	0.000	0.200	0.000	0.400	0.000	0.000	0.400	0.000	0.000
SV5					1	0.333	0.000	0.200	0.000	0.000	0.000	0.400	0.250	0.000	0.200	0.500	0.400	0.167	0.333	0.167	0.333	0.000
SV6						1	0.000	0.000	0.167	0.200	0.333	0.286	0.167	0.167	0.333	0.333	0.286	0.500	0.000	0.125	0.200	0.400
SV7							1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SV8								1	0.000	0.000	0.200	0.167	0.000	0.250	0.000	0.200	0.167	0.167	0.333	0.400	0.000	0.000
SV9									1	0.000	0.250	0.200	0.333	0.000	0.250	0.000	0.200	0.200	0.000	0.200	0.000	0.333
SV10										1	0.333	0.000	0.000	0.500	0.000	0.333	0.000	0.250	0.000	0.000	0.000	0.500
SV11											1	0.167	0.000	0.250	0.200	0.200	0.167	0.400	0.000	0.167	0.000	0.667
SV12												1	0.200	0.000	0.400	0.400	0.143	0.333	0.250	0.000	0.000	0.200
SV13													1	0.000	0.000	0.250	0.500	0.200	0.000	0.200	0.000	0.000
SV14														1	0.000	0.250	0.000	0.500	0.000	0.200	0.000	0.333
SV15															1	0.000	0.167	0.167	0.000	0.167	0.333	0.250
SV16																1	0.167	0.400	0.333	0.000	0.000	0.250
SV17																	1	0.143	0.000	0.600	0.250	0.000
SV18																		1	0.000	0.143	0.000	0.500
SV19																			1	0.000	0.000	0.000
SV20																				1	0.250	0.000
SV21																					1	0.000
SV22																						1

Cophenetic Correlation Coefficient = 0.736388

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, 2022

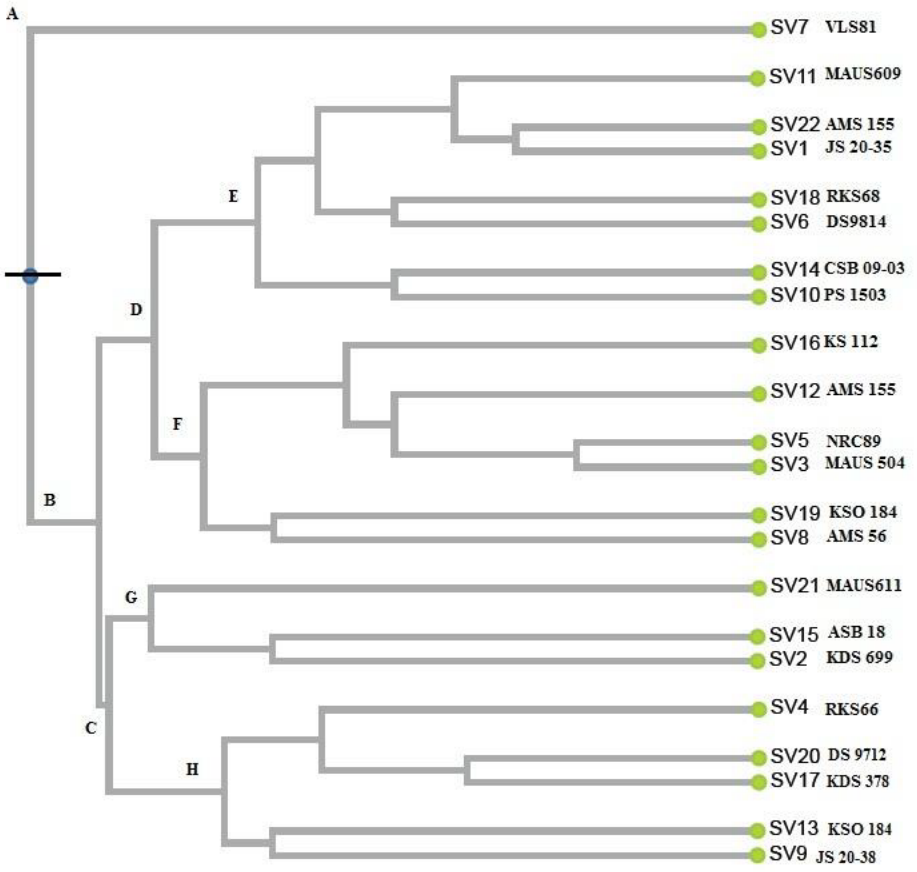

Figure 7 UPGMA Dendrogram of Soybeans Germplasm based on SSR Marker

4. Discussion

Soybean is an important legume crop in the world's cultivation of oilseed scenario, because of its high productivity, profitability, and vital contributions. Soybean has a role in maintaining the fertility of the soil studied by Lala, et. al., 1999. Jain R.K. et. al. suggested that the Soybean is an oilseed crop which having 20% oil and 38 - 43% protein has contained many nutrients such as meat and fish protein. The genetic variances in 24 genotypes of soybean and their observations on 9 characters were recorded. This study is comparable to the findings of Jain, et. al., concerning the diversity of soybeans in Central India.

Molecular markers like SSR are advantageous for the identification of diverse genotypes over the conventional approach (Thompson et. al., 1998). SSR markers have been widely applied in the genetic diversity studies of the soybean germplasm. (Fuet. al., 2007 and Ristova et. al., 2010). In the present study, all 10 markers were polymorphic and produced unique allelic profiles for the 22 Indian Soybean genotypes. The genetic diversity observed in the present study is comparable to those reported by Ristova, et. al. 2010, where numbers of alleles

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, 2022

ranged from two to seven with an average of 3.4 alleles per locus. The highest allelic variability was observed in Satt 114 with 7 alleles and Satt 256 with 4 alleles. Satt 656, Satt 177, Satt 299, Satt 460, Satt 038, Satt 656, Satt 396, and Satt 178 were the least variable locus each with three (3) alleles whereas Satt070 with 2 alleles.

The SSR marker profiles of 22 Soybean genotypes were generated by primer Satt 256, Satt 299, Satt460, Satt 114, and Satt 070. The higher the PIC value, the more informative is the SSR marker. The PIC values of markers ranged from 0.3729 to 0.8274 with an average of 0.5426. Thus, this result provides evidence about the reliability of the use of SSR markers for the analysis of genetic diversity in crop plants. The SSR markers used in this study could reveal the amount and extent of genetic diversity present in soybean cultivars. The markers identified in this study along with the diverse parents identified can be used in future breeding to improve the genetic quality of soybeans (Zhang et. al., 2013).

In the current study, the dendrogram showed 3 major clusters. Cluster A consisted of a VL S81 variety of soybeans. Cluster B consisted of 21 varieties of soybean cluster B further grouped into two clusters C and D. Genetic variance and genetic distance determination revealed results as Masud, et. al., 2014 and Prysiazhniuk, et. al., 2019that obtained a correlation between genetic distances by using DNA markers.

5. Conclusion

There is moderate genetic variation among released and elite soybean genotypes of Uganda. The SSR marker i.e. Satt411 was highly informative and could be useful and this is used as a tool to determine the genetic variability between the soybean accessions and the study about phylogenetic relationship means evolutionary relationship. As a long-term breeding strategy, the Soybean Breeding program can believe the use of pre-breeding and other novel techniques of biotechnology such as mutation breeding or gene editing by CRISPR that are known to enhance genetic diversity.

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