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

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Abstract

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.

Keywords
Soybean, Molecular Characterization, Genetic Diversity, SSR markers, Allele

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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 MgCl2, 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 94⁰C followed by 35 cycles of DNA denaturation at 94⁰C for 30 seconds, primer annealing at 50-55⁰C for 30 seconds and DNA extension at 72⁰C for 7 minutes was performed after 35 cycles. Amplified PCR products was
separated on 2.0% of agarose gel at a voltage 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 (°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 TAC AAT TGA TGC CAT AAA T         | 55                            |
| Satt 270  | GCG CAG TGC ATG GTT TTC TCA           | TGT GAT GCC CCT CT T                 | 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 TTC TCA AGT       | AAC ATC CAA AGA AAT GTG TTC ACA A    | 55                            |
| Satt 309  | GCG CCT TAA ATA AAA CCC GCA 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 GTT TTT GTT CTT A     | GCG CTA TTC CTA 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 CATCAA TCA CA            | AAA CCC ACC CTC AAA AAT AAA A        | 52                            |
| Satt 598  | CAC AAT ACC TGT GGC GTI TAT ACT AT    | CGA TTT GAA TAT ACT TAC GGT 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 TTA GTG TGG CTA AG        | GCG CAC GTT GAT TTT TTT ATA GTA A    | 52                            |
| Satt 671  | GCG AGA AAT GAG ATA ATG GTT GAT A     | GCG TAA TAC 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 TTA TGG AGC AAA ATTC      | 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 CTC CTA GAA      | GGA TTT CAA AAG ATG AAC ACA GA       | 55                            |
| Satt 523  | GCG CTT TTT CGG CTG TTA TTA TTA ACT  | GCG ATT TCT TCC TGG AAG AAT TTT CTG  | 55                            |
| Satt 353  | GCG AAT GGG AAT GCC TTC TTA TTC TA    | CAT ACA CGC ATT GCC TTC TTT GAA     | 55                            |
| Satt 414  | GCG TCA TAA TAA TGG 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 CCC CAT TGG CTT TCT 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 TTA TTT AAA ACAA AACA CACA AC | GCG ACA TAT TGG 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 TAA CCC AGG GAC TTA CT         | 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 T        | 50                            |
| Satt 160  | CAT CAA AAG TTT ATA ACG TGT AGA T     | TCC CAC ACA GTT TCC 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 ATG ATA AAT GCA ATC ACT TAA AAA G | GCG GCG GTA ATG TAC GTT GAG A | 58 |
| Sat_366  | GCG GAC ATG GTA CAT CTA TAT TAC GAG TAT T | GCG GCA CAA GAA CAG ACG 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 CTG 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 CGA 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 TGG 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                |
| 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                     |                                |

Table 3: Details of five unique SSR alleles identified
**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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