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Full Length Research

Molecular characterization of soybean (*Glycine max* (l.) Merril) genotypes using SSR markers

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ABSTRACT: Six SSR primers were used to amplify genomic deoxyribonucleic acid (DNA) from 10 soybean genotypes, after which the level of diversity among the genotypes were elucidated based on marker polymorphism. Seventeen alleles with a mean value of 2.8 alleles per locus were detected. The polymorphic information content (PIC) among genotypes ranged between 0.16 to 0.60 with an average PIC of 0.45 and a gene diversity of 0.51. A phylogenetic tree was produced from the allelic diversity data, which clustered the genotypes into two groups. The major cluster was divided into two sub-groups, each consisting of 3 genotypes, while the minor cluster was divided into three sub-groups. Findings on the single sequence repeat (SSR) primers used in the study indicated high levels of polymorphism, which is an important attribute for ascertaining genetic diversity. The utility of the SSR marker system and reliability of DNA characterization are both useful tools for assessing soybean genetic diversity.

Keywords: Characterization, genetic diversity, polymorphism, soybean, SSR.

INTRODUCTION

Soybean *Glycine max* (L.) Merril is a very important oil seed which is grown around the world. It belongs to the bean family Fabaceae the genus *Glycine* (Singh, 2017). It is grown basically for its seed which is rich in oil and protein and is often referred to as the 'Miracle crop' or 'Golden bean' due to its numerous uses and economic importance. It is used in the production of a nutritious drink called 'soya milk' and '*awara'* (soybeans cake) in Nigeria. It is also used as a component of meals for infants and also as a very vital ingredient in the formulation of feeds for poultry and fishery. The oil is used as cooking oil and as the base for shortening, margarine, salad dressings and mayonnaise (Pratap *et al*., 2012). Lecithin extracted from the oil is also used in the manufacture of health and nutrition products, cosmetics and industrial coatings (List, 2015). For effective breeding programs, information concerning the extent and nature of genetic diversity or variation within a crop species is essential as this knowledge may help improve desirable traits and can be used by plant breeders to determine suitable parents (Jain *et al*., 2017). Several methods have been employed in the determination of genetic diversity among species such as morphological and biochemical methods but they have limitations such as influenced by environmental conditions (Chauhan *et al*., 2015). The use of PCR-based molecular markers is seen as an ideal way to conserve plant genetic resources, distinguish between genotypes and determine the breeding performance of species. Single sequence repeats (SSRs) also known as microsatellite markers consist of tandem repeats of nucleotides, with short sequences. They have advantageous attributes, which inform their use as tools for genetic diversity determination in species such as their co-dominant and multi-allelic nature, ability to detect higher levels of polymorphism, easy scorability, simplicity and reproducibility (Chauhan *et al*., 2015). Zhang *et al*.

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(2013) noted that SSR markers have been widely employed in studies of genetic diversity in soybean because they are of high advantage in the identification of variability among genotypes. Therefore, the objective of this study was to determine the genetic diversity among the soybean genotypes using SSR markers.

MATERIALS AND METHODS

Ten (10) soybean genotypes, TGx 1987-62F, TGx 1835, TGx 1955, TGx 1951-3F, TGx 1904-6F, TGx 1448-2E, TGx 1987-10F of International Institute of Tropical Agriculture (IITA) origin and NCRISOY 56, NCRISOY 16, JG of National Cereal Research Institute (NCRI) origin were sown on the field, at the Teaching and Research farm of the School of Agriculture and Agricultural Technology, Federal University of Technology, Gidan-Kwano campus located at longitude 09.52935ºN and latitude 006.45025ºE, 234 m above sea level, Minna, Niger state, in the Southern Guinea Savanna agro-ecological zone of Nigeria. The laboratory work, DNA extraction and primer selection were done at the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo state, Nigeria.

DNA extraction and primer selection

Leaf samples of 10 soybean genotypes at 4 weeks after sowing were collected for DNA extraction. Deoxyribonucleic acid extraction from each soybean genotype was carried out using Dellaporta DNA extraction protocol (Dellaporta *et al.*, 1983). A total of six Simple Sequence Repeats (SSRs) primer pairs (Table 1), selected across the integrated linkage map of soybean (Cregan *et al*., 1999) were used and synthesized by Integrated DNA Technologies. The SSR markers that were selected had an (ATT) motif due to their abundance and polymorphic nature in soybeans and their easily interpretable allele patterns (Narvel *et al*., 2000).

Polymerase Chain Reaction (PCR)

PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA). Reaction cocktail used for all PCR per primer set included (Reagent Volume µl) - 5X PCR SYBR green buffer (2.5), $MgCl_2$ (0.75), 10pM DNTP (0.25), 10 pM of each forward and backwards SSR primer (0.25), 8000 U of taq DNA polymerase (0.06) and made up to 10.5 with sterile distilled water to which 2 µl template was added. A touch down PCR profile was used consisting of an initial denaturing for 5 minutes at 94°C followed by 9 cycles consisting of a 15 seconds denaturing at 94°C, 20 seconds annealing at 65°C and 30 seconds extension at 72°C followed by 35 cycles consisting of a 15 seconds denaturing at 94°C, 20 seconds annealing at 55°C and 30 seconds extension at 72°C. The reaction was terminated at 72°C for 7 minutes and kept on hold at 10 °C.

Integrity and Gel electrophoresis

The integrity of the amplified fragment was checked on a 2% Agarose gel. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 2% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool for 5 minutes and stained with 3 µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). The gel was swirled gently for even distribution of the ethidium bromide. A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel, after which 6 µl of each PCR product was loaded into the wells after the 100 bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120 V for 45 minutes visualized by ultraviolet trans-illumination and photographed with a digital camera. The sizes of the PCR products were estimated by comparison with the mobility of a 100 bp molecular weight ladder that was ran alongside experimental samples in the gel and the presence or absence of a binding pattern was scored for each primer and used for analysis.

Data scoring and analysis

The PCR products from SSR amplification were analyzed by scoring qualitatively for presence or absence of amplification band with 1 representing presence and 0 representing absence. This was used to generate a score matrix on which basis the genetic similarity between the genotypes was estimated. This matrix was imported into the Power marker V3.25 (Chauhan *et al*., 2015) and used to estimate the major allelic frequency, gene diversity and polymorphism information content. Phylogenetic analysis was estimated using the minimum likelihood estimation as provided by the Molecular Evolutionary Genetics Analysis Version 6.0 (MEGA6) software (Tamura *et al*., 2013).

RESULTS AND DISCUSSION

In this study, all the 6 SSR markers used for the analysis of genetic diversity amplified scorable loci with none being monomorphic as shown in Table 2. This supports the findings of Chauhan *et al*. (2015) who characterized 48 soybean genotypes and found all the 21 SSR primers used

Table 1. SSR primers and sequences.

Primer code	Forward sequence	Reverse sequence
satt123	TTC AAC CAT TCA AAC ATG	AAT TGA AAA GAT GTG AAA CAT
satt135	CAC GGA TTT TAA ATC ATT ATT ACA T	TTC CAA TAC CTC CCA ACT AAC
satt294	GCG GGT CAA ATG CAA ATT ATT TTT	GCG CTC AGT GTG AAA GTT GTT TCT AT
satt309	GCG CCT TCA AAT TGG CGT CTT	GCG CCT TAA ATA AAA CCC GAA ACT
satt581	CCA AAG CTG AGC AGC TGA TAA CT	CCC TCA CTC CTA GAT TAT TTG TTG T
satt609	CCT ACG CAA GTA ACA TTG GTT GTC AT	GCG GCT AAA ACG TAT TAA ATT AAG A

Table 2. Frequency of major allele, allele number, availability, gene diversity, and PIC of SSR primers in soybean.

Marker	Major allele frequency	Allele No	Gene diversity	PIC
Satt123	0.7	3	0.46	0.4102
Satt135	0.4	3	0.66	0.5862
Satt294	0.4	3	0.66	0.5862
Satt309	0.9	2	0.18	0.1638
Satt581	0.4	4	0.66	0.5958
Satt601	0.7	2	0.42	0.3318
Mean	0.58	2.8	0.51	0.45

PIC = polymorphic information content.

for the study to be polymorphic. The high rate of polymorphism (100%) detected in this study can also be compared with the findings of Tantasawat *et al*. (2011) and Giriraj *et al*. (2015), where similar results were obtained. This high rate of polymorphism in SSR locus may be ascribed to the selected set of primers used for the study, which amplified regions of high polymorphism among the genotypes. The genetic diversity which shows the probability that two alleles selected at random are different from others in the population, ranged between 0.18 (satt309) to 0.66 (satt135, satt294 and satt581), with a mean value of 0.51. This is considered to be higher than the results obtained by Anchal *et al*. (2015) where the genetic diversity ranged between 0.05 to 0.61 with a mean value of 0.23 and the low level of genetic diversity was reported to be attributed to direct introduction, selection from introduced germplasm and single cross hybrids in the soybean breeding programs. Anchal *et al*. (2015), therefore proposed the inclusion of more diverse soybean germplasms in breeding programs to boost genetic diversity.

A total of 17 alleles were detected among all the 10 genotypes in the study. The banding patterns of the ten soybean amplified by the six SSR primers are shown in Plate I to Plate VI. The highest allele number was recorded for satt581 (4), the lowest number of alleles were recorded in satt309 and satt601 (2), while satt123, satt135 and satt294 fell in-between, having 3 alleles each. However,

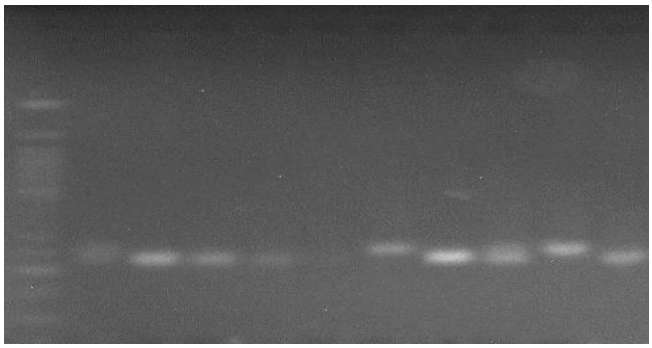

Plate I. Gel image for satt123 primer.

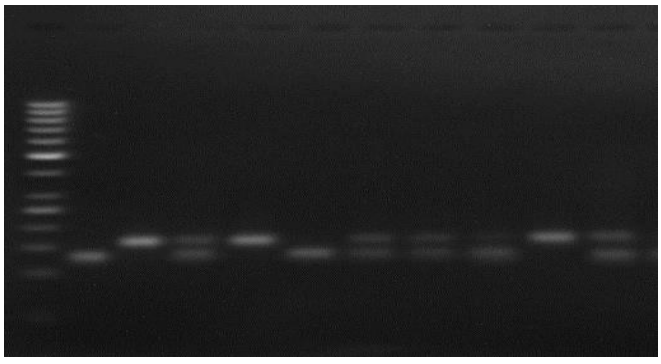

Plate II. Gel image for satt135 primer.

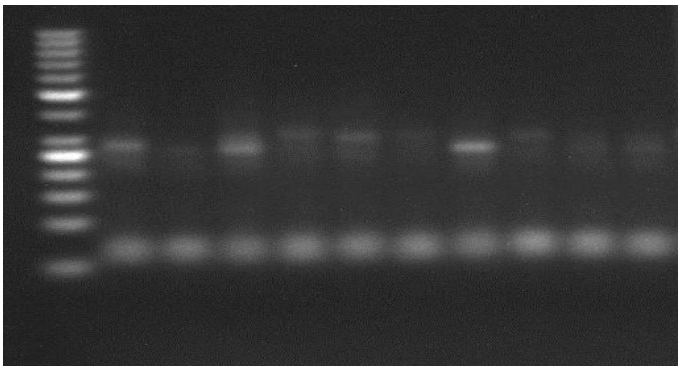

Plate III. Gel image for satt294 primer.

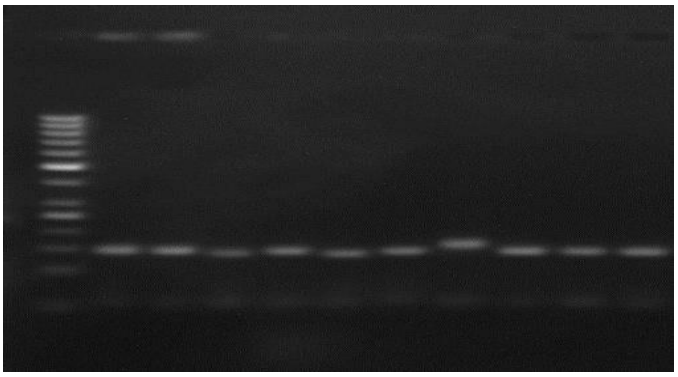

Plate IV. Gel image for satt309 primer.

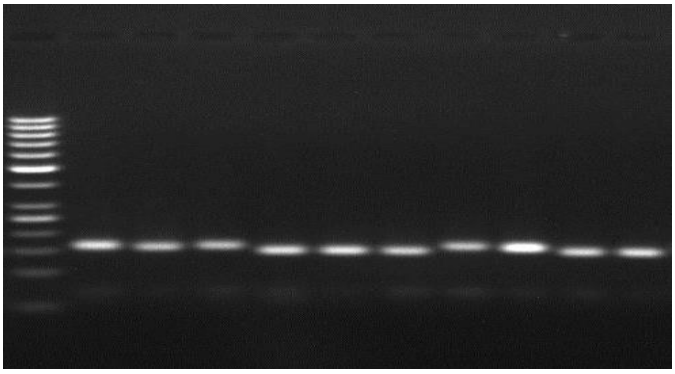

Plate V. Gel image for satt581 primer

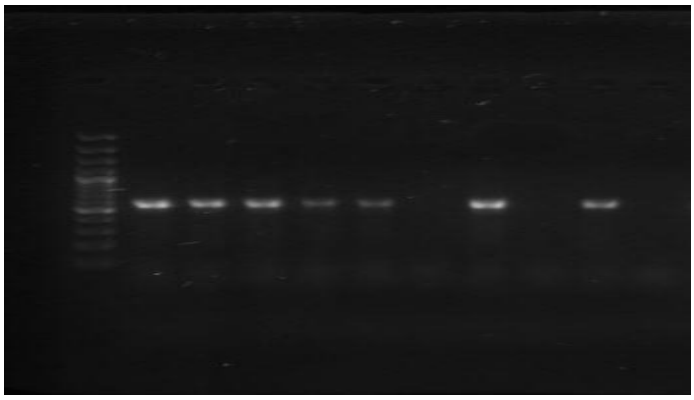

Plate VI. Gel image for satt601 primer.

low mean values for allele number and polymorphic information content (Table 2) were observed. The allele number for each SSR locus varied from 2 to 4 alleles with a mean value of 2.8. The genetic diversity for each primer was evaluated by the value of polymorphic information content (PIC). The highest PIC value (0.5958) was recorded for satt581 while the lowest was recorded for satt309 (0.1638) with a mean PIC value of 0.45. The major allele frequency was lowest in satt135, satt294 and satt581 (0.4), while the highest (0.9) was recorded for satt309 with a mean value of 0.58. This indicates low diversity in the set of soybean genotypes considered (Giriraj *et al*., 2015) but agrees with the study of Hisano (2008), who observed the same mean value for number of alleles (2.8) and a comparable mean PIC value of 0.40.

The PCR products were scored qualitatively for presence or absence of amplification with 1 representing presence and 0 representing absence as shown in Table 3. Phylogenetic analysis was estimated after which a dendrogram was generated (Figure 1). The allelic diversity data was used to produce a dendrogram (phylogenic tree) to explain the genetic relationships among the tested genotypes (Figure 1). The maximum similarity co-efficient (60%) was found between TGX 1951-3F versus TGx 1987-62F, JG, TGx 1835-10E and TGx 1904-6F versus TGx1448-2E, this was in agreement with Chauhan *et al.* (2015) findings, who characterized 48 soybean varieties using 21 SSR markers, with at least one primer from each linkage group. Additionally, the minimum similarity coefficient (40%) was recorded between genotype TGx 1955, NCRI SOY 56 and NCRI SOY 16 versus TGx 1987-10F. The cluster analysis divided the total 10 genotypes into two groups: 6 genotypes in major group and 4 in minor group respectively. The major group was further subdivided into two subgroups. Each of these subgroups consisted of three genotypes with TGx 1951-3F, TGx 1987-62F and JG making up the subgroup A and subgroup B comprising of TGx 1835-10E, TGx 1904-6F and TGx 1448-2E. The minor group was divided into three subgroups, with TGx 1955 and NCRI SOY 56 found to be distinctly different from NCRI SOY 16 and TGx 1987-10F. This indicates diversity in the set of soybean genotypes considered (Giriraj *et al*., 2015) but in agreement with the findings of Hisano (2008), who observed similar trend.

Table 3. Data scoring of SSR primers.

Level-1	Satt123	Satt135	Satt294	Satt309	Satt581	Satt601
NCRI SOY 56	1/0	0/1	1/0	1/0	1/0	1
TGX 1955	1/0	1/0	0/0	1/0	1/0	1
NCRI SOY 16	1/0	1/1	1/0	1/0	1/0	1
JG	1/0	1/0	0/1	1/0	0/1	1
TGX 1951-3F	0/0	0/1	0/1	1/0	0/1	1
TGX 1904-6F	0/1	1/1	0/0	1/0	1/1	0
TGX 1987-10F	1/0	1/1	1/0	0/1	1/0	1
TGX 1987-62F	1/0	0/1	0/1	1/0	0/0	0
TGX 1448-2E	0/1	1/0	0/0	1/0	0/1	1
TGX 1835-10E	1/0	1/1	0/0	1/0	0/1	0

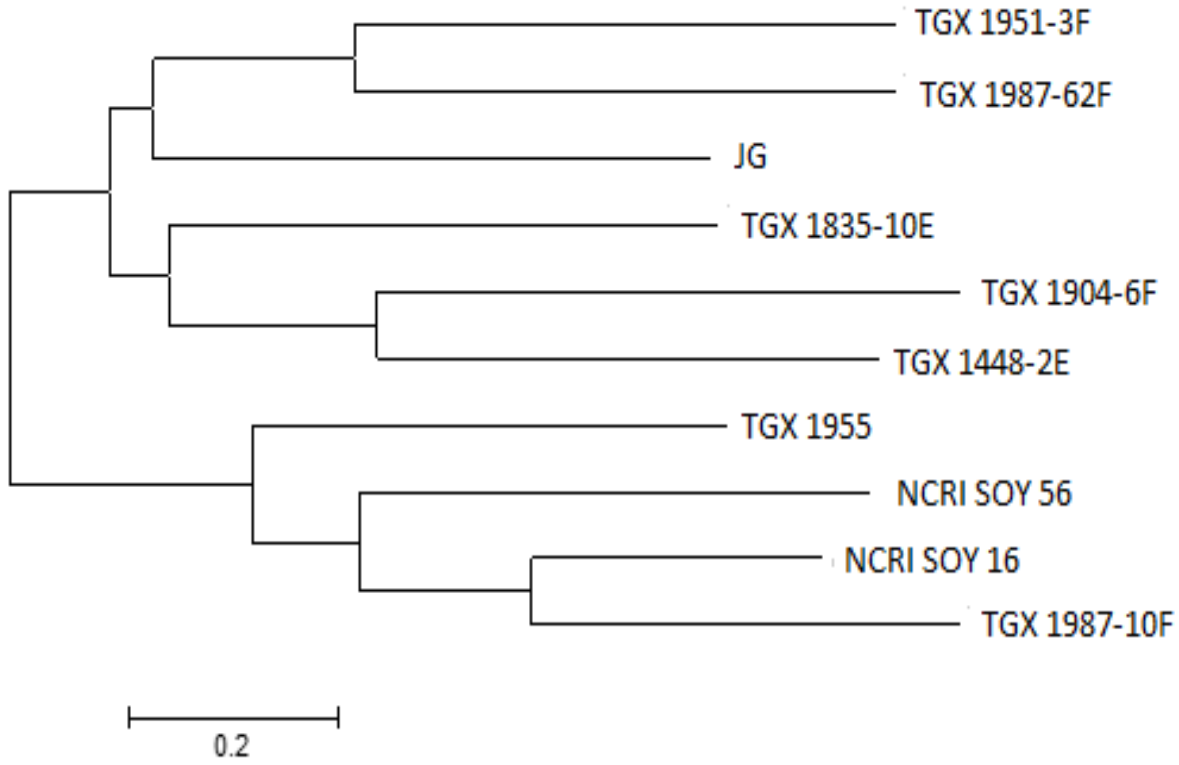

Figure 1. UPGMA dendrogram of soybean genotypes based on SSR markers.

Conclusion

The SSR primers used in the study exhibited high levels of polymorphism, which is an important attribute for ascertaining genetic diversity, hence it serves as an effective and reliable tool for analysis of genetic relationship among cultivars, identifying soybean cultivars and selection of better soybean lines. Based on the primers used, the ten soybean genotypes were also classified into two major groups. Six genotypes (TGx 1951-3F, TGx 1987-62F, JG, TGx 1835-10E, TGx 1904-6F and TGx 1448-2E) where found to be genetically similar and classified in group I, while the other four genotypes (TGx 1955, NCRISOY 56, NCRISOY 16 and TGx 1987-10F) made up group II.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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