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Genetic Diversity in Okra Genotypes Revealed by Simple Sequence Repeats (SSR) Markers

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ABSTRACT

This research was conducted to determine the molecular diversity and genetic analysis of fruit yield related characters in Okra genotypes, Abelmoschus esculentus (L.) Moench. The 8 Okra genotypes were obtained from the Okra germplasm collection of National Center for Genetic Research and Biotechnology (NACGRAB) Ibadan, Oyo State, Nigeria. The 8 SSR markers utilized for this study were highly polymorphic recording average allele number of 6.63 and average polymorphic information content of 0.76. The primers categorized the Okra genotypes into 3 clusters. 1, 4 and 3 Okra genotypes respectively. Three of the Okra genotypes; NCB00303, NCB00396, NCB00466 stand out in terms of Number of fruits per plant. Hence, they could be selected as promising genotypes with respect to donor parents in future Okra breeding programmes.

Keywords

Okra, Vegetables, Genomic DNA Extraction.

Introduction

Okra [Abelmoschus esculentus (L.) Moench] is an important vegetable. Okra is a member of the malvaceae family, which includes fiber crops such as cotton (Gossypium spp) and kenaf (Hibiscus cannabianus) [1]. The origin of okra remains unclear but centers of genetic diversity include West Africa, India and Southeast Asia [2]. Okra is a traditional vegetable crop in many tropical, subtropical and Mediterranean countries [3]. It is widely distributed and cultivated in the tropics, sub tropics, and warmer portions of the temperate region of the world on a varying scale [3]. It is mainly grown for its young immature fruits and consumed as a vegetable, raw, cooked, or fried. Also, okra fruit has high nutritional value, which contains, carbohydrates, fats, fibres, oil, mineral and vitamins viz., B1, A and C [4]. Okra requires a long, warm and humid growing period. It can be successfully grown in hot humid areas [5]. Okra has good nutritional value, particularly rich in vitamins (30 mg/100 g), calcium (90 mg/100 g) and iron (1.5 mg/100 g). It is an excellent source of iodine and useful for the control of goiter disease [6]. Knowledge of genetic diversity of a species has an important impact on the improvement of crop productivity as well as the conservation of genetic resources. Study of phenotypic and genetic diversity in germplasm collection is important for germplasm conservation [7]. In addition, the characterization of much diversified materials with molecular markers offers a unique opportunity to define significant marker trait association of biological and agronomic interest but these markers are highly influenced by environmental factors [8]. The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the most significant developments in the field of molecular genetics [9]. Molecular markers have proven to be powerful tools in the assessment of genetic variation and evaluation of genetic relationships within and among species. Various types of molecular markers differing in their principles and methodologies are available such as AFLP, SSR, RFLP and RAPD. The present research was carried out to characterize okra genotypes at molecular level using SSR markers and to measure genetic diversity and relatedness among studied okra genotypes.

Materials and Methods

The okra genotypes utilized for this study were obtained from the okra germplasm collection of National Center for Genetic

Research and Biotechnology (NACGRAB), Ibadan, Oyo, - State, Nigeria.

Table 1: Names and source of Okra genotypes, *Abelmoschus esculentus*.

Names	Source
NCB00303	NACGRAB
NCB00322	NACGRAB
NCB00323	NACGRAB
NCB00324	NACGRAB
NCB00369	NACGRAB
NCB00396	NACGRAB
NCB00466	NACGRAB

Genomic DNA Extraction

About 2 g of leaves from 2 weeks old of potted cowpea was ground after being surface sterilized with ethanol and 1000µl of freshly prepared modified CTAB extraction buffer (100mMTris-HCl, pH 8.0; 20mM EDTA, pH 8.0; 1.4M NaCl; 2% CTAB; (just before use) was added in mortar and pestle. The resultant mixture was homogenized and incubated in 60°C water bath for 30min. Following the incubation period, the tube was transferred into centrifuge at 12,000RPM for 10minutes. The supernatant was transferred into a new sterilized eppendorf tube, then 10µL of RNases H solution was added and incubated at 37°C for 5minutes. It was allowed to cool for 7 min and 1000µL chloroform: isoamylalcohol (24:1) was added in the tubes and centrifuged at 12,000 rpm for 5minutes. The supernatant recovered after centrifugation was transferred into new tubes and up to 500µL Isopropanol was added and kept in -10°C freezer for 30minutes for DNA precipitation. The pellet was collected by centrifugation at 12,000 rpm for 2 min and washed with 500µL of chilled 70% ethanol. Pellet was air-dried until no further trace of ethanol. An average of 40 µL nuclease free water T.E. buffer was added to elute the DNA and stored at -10°C.

DNA Quantification

U. V. spectrophotometer was blanked using TAE buffer. 2µL of the dissolved DNA pellet was transferred into micro eppendorf tube and 48µL of TAE buffer was added into the tube. The solution was mixed and measured on the U. V. spectrophotometer. The result of the U.V spectrophotometer was recorded.

Gel Electrophoresis Run

5µL of dissolved DNA was introduced into micro eppendorf tube and 2µL of loading dye was added. Agarose preparation: 0.8% of agarose was weighed into a glass beaker and TAE buffer was added into the beaker, then mixed thoroughly. The mixture was microwaved for 2minutes. Gel casting: 2µL of save view was added into the gel and casted on the gel tray containing the comb, then allowed to solidified. After solidification, the comb was removed and the solidified gel was transferred unto the tank containing TAE buffer. The 3.5µL of the prepared DNA was introduced into the gel well and was charged at 120volts for 10minutes. The gel was viewed under U. V illuminator and the result was captured.

Viewing the DNA by the Gel Documentation Apparatus

After electrophoresis finished, gloves were used to take the gel and placed it in the gel documentation chamber, the camera was operated and image was adjusted on it. The chamber was closed and the U.V. light was turned on. The picture was viewed on the camera screen and captured.

SSR analysis

SSR primers developed by Schafleitner et al. were used in this study. PCR reaction for SSR was performed in 10µl volume containing 20 ng template DNA and PCR master mix (NEB). The PCR reaction conditions were an initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 30s, 55°C for 30s, 72°C for 30s, and a final extension at 72°C for 10 min. The amplified SSR products were separated on 6 % agarose gel electrophoresis and were visualized by silver staining with EZ-Vision. The gels visualized under blue light using Bluebox™ (USA).

Data Analysis

The statistical analysis was conducted by scoring SSR bands as codominant. These bands were considered as polymorphic when they were absent in some samples. Percent polymorphism for each marker was generated by the formula: (Number of polymorphic bands/Total number of scored bands) X 100. The polymorphic information content (PIC), a degree of polymorphism, was obtained with the Polymorphic Information Content (PIC) calculator. To analyze the diversity, the amplicons were scored based on band width and the pair-wise genetic similarity between genotypes was generated through Jaccard's co-efficient. PAST 3 was subjected to generate dendrogram using the Unweighted Pair Group Method Average (UPGMA) clustering. The computer program BOOTSTRAP was used to examine the robustness of the dendrogram nodes with 100 bootstraps.

Results and Discussion

The names and source of the okra genotypes are presented in Table 1. The names and sequences of the SSR markers are presented in Table 2. A set of primer pairs were pre – selected based on their ability to PCR amplify SSRs in okra genotypes to examine the level of genetic diversity and relatedness among the okra genotypes. The Allele Number and Polymorphic Information Content (PIC) of the SSR primers used is presented in Table 3. A total of 53 alleles at 8 loci could be scored. The number of alleles detected per primer pairs varied from 3 to 8 with an average of 6.63 alleles. Marker OKRA104, OKRA105, OKRA108 and OKRA111 detected the highest alleles number (8) and with the allele percentage (15.09%) while the lowest allele number (3) was detected by OKRA112 and allele percentage (5.66%). On the average, 62.5% of the total SSR markers detected allele number above 6.63 Polymorphic Information Content (PIC) ranged from 0.37 to 0.86 with a mean of 0.76. The highest Polymorphic Information Content (PIC) percentage (14.10%) was detected by marker OKRA104, OKRA105, OKRA108 and OKRA111 while the lowest Polymorphic Information Content (PIC) percentage (6.07%) was observed in OKRA112. On the average, 37.5% of the markers recorded Polymorphic Information Content (PIC) percentage value below 12.50.

The estimates of the allele number, major allele frequency and gene diversity for the primers used are presented in Table 4. The number of major allele frequency per primer pairs ranged from 0.12 to 0.75 with an average of 0.28. Marker OKRA112 recorded the highest major allele frequency 0.75 while marker OKRA104, OKRA105, OKRA108 and OKRA111 recorded the lowest major allele frequency (0.12). The number of alleles detected per primer pairs varied from 3 to 8 with an average of 6.63 alleles. Marker OKRA104, OKRA105, OKRA108 and OKRA111 detected the highest alleles number (8) while the lowest alleles number (3) was detected by OKRA112. The gene diversity ranged from 0.40 to 0.87 with an average of 0.78. On the average 62.5% of the primers recorded gene diversity value above 0.78.

The SSR primers profile across the Okra genotypes are presented in Plates 3a to 3h. Maximum polymorphism was observed in OKRA104, OKRA105, OKRA108 and OKRA111 and it was minimum in primer OKRA112. OKRA108 and OKRA111 and it was minimum in primer OKRA112.

Table 2: Names and Primer Sequence of the SSR Markers Used for the Analysis of 8 Okra Genotypes, *Abelmoschus Esculentus*.

Name	Forward	Tm	Name	Reverse	Tm
OKRA103 F	5′GAATTCGATTCCAATACAGG 3′	47.68	OKRA103 R	5′ TCGTCGTCTTCATTTCTCTT 3′	47.68
OKRA104 F	5′ CGGTAAATCTTGTCTCTTGC 3′	49.73	OKRA104 R	5′ TATAGGAAAACCCCCAAGAT 3′	47.68
OKRA105 F	5′ CCTCAACGAGGAGTAGAAGA 3′	51.78	OKRA105 R	5′ CCTTCATCATAATCCATCTAGG 3′	51.11
OKRA108 F	5′ AAGAAGGAGAAGAGGGAATG 3′	49.73	OKRA108 R	5′ TAAACCGTCTAGGAACTCCA 3′	49.73
OKRA109 F	5′ TTTCCCTAATGAGTGGACC 3′	48.93	OKRA109 R	5′ GGGTCTGTTTTGTTGTTGTT 3′	47.68
OKRA110 F	5′ GGCAACAACAGTTCTCCTT 3′	48.93	OKRA110 R	5′ AATTGGGGTTAGTGACGATA 3′	47.68
OKRA111 F	5′ CATTTTAAGGAGCGAGTGTC 3′	49.73	OKRA111 R	5′ CTCTTCCTCAACAAACCAG 3′	49.73
OKRA112 F	5′ CTCAATTGGATTGGATGAGT 3′	49.73	OKRA112 R	5′ CCTCTCGAACTGAGAAAGAAA 3′	49.73

Table 3: Allele Number, Allele Percentage and Polymorphism Information Content (Pic) for the Primers Used.

MARKER	ALLELE NO	ALLELE %	PIC	PIC %
OKRA103	6.00	11.32	0.75	12.35
OKRA104	8.00	15.09	0.86	14.10
OKRA105	8.00	15.09	0.86	14.10
OKRA108	8.00	15.09	0.86	14.10
OKRA109	7.00	13.20	0.82	13.50
OKRA110	5.00	9.43	0.71	11.68
OKRA111	8.00	15.09	0.86	14.10
OKRA112	3.00	5.66	0.37	6.07
MEAN	6.63	12.50	0.76	12.50

Table 4: Major allele frequency, allele number and gene diversity for the primers used.

MARKER	ALLELE NO	MAJOR ALLELE FREQUENCY	GENE DIVERSITY
OKRA103	6.00	0.37	0.78
OKRA104	8.00	0.12	0.87
OKRA105	8.00	0.12	0.87
OKRA108	8.00	0.12	0.87
OKRA109	7.00	0.25	0.84
OKRA110	5.00	0.37	0.75
OKRA111	8.00	0.12	0.87
OKRA112	3.00	0.75	0.40
MEAN	**6.63**	**0.29**	**0.78**

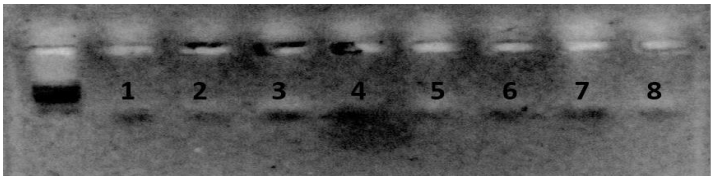

Plate 3a: Gel Electrophoresis. DNA bands amplified by OKRA103 marker across 8 Okra genotypes.

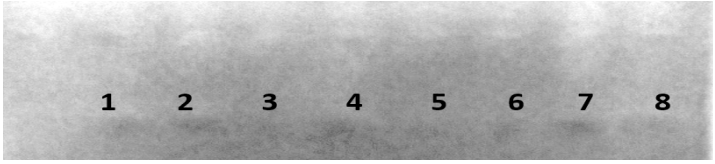

Plate 3b: Gel Electrophoresis. DNA bands amplified by OKRA104 marker across 8 Okra genotypes.

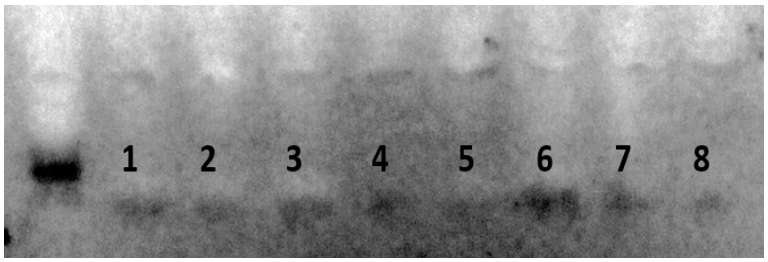

Plate 3c: Gel Electrophoresis. DNA bands amplified by OKRA105 marker across 8 Okra genotypes.

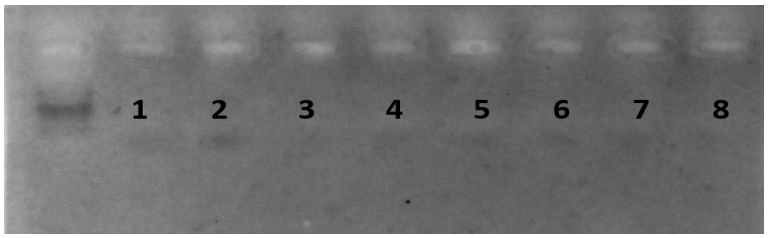

Plate 3d: Gel Electrophoresis. DNA bands amplified by OKRA108 marker across 8 Okra genotypes.

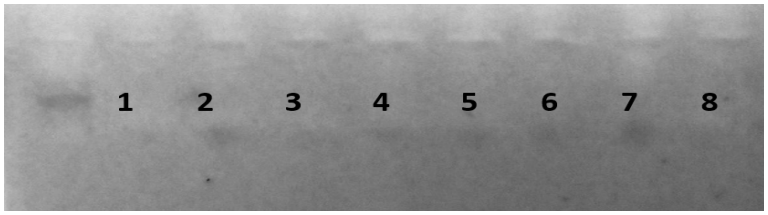

Plate 3e: Gel Electrophoresis. DNA bands amplified by OKRA109 marker across 8 Okra genotypes.

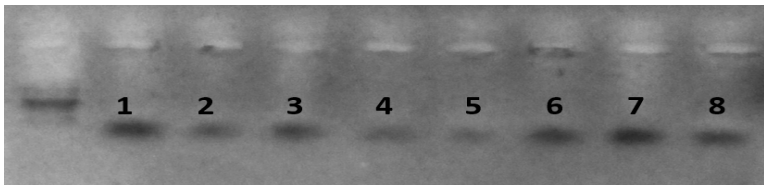

Plate 3f: Gel Electrophoresis. DNA bands amplified by OKRA110 marker across 8 Okra genotypes.

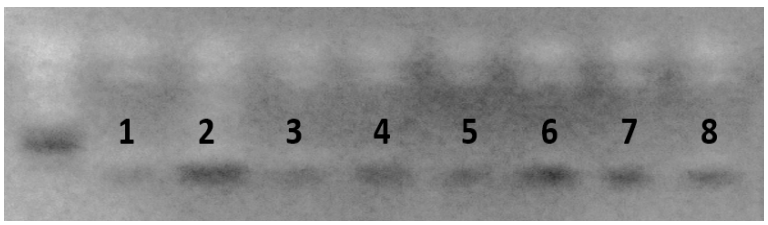

Plate 3g: Gel Electrophoresis. DNA bands amplified by OKRA111 marker across 8 Okra genotypes.

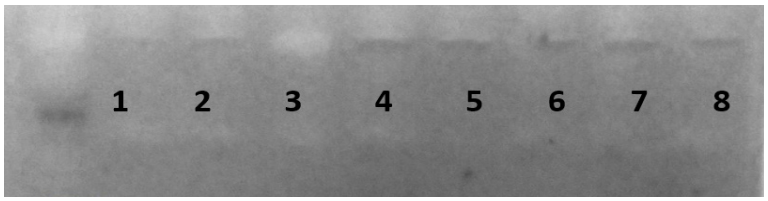

Plate 3h: Gel Electrophoresis. DNA bands amplified by OKRA112 marker across 8 Okra genotypes.

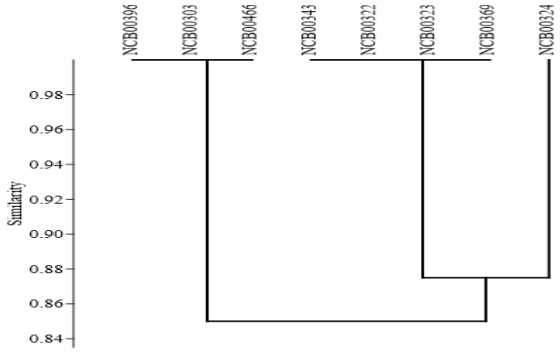

Figure 1: UPGMA based cluster analysis of 8 Okra genotypes using SSR markers.

A dendrogram based on UPGMA cluster analysis of the SSR data among the Okra genotypes is shown in Figure 1. The dendrogram separated the Okra genotypes into 2 clearly distinct groups with the first group subdivided into 2 clusters A and B and the second group referred to cluster C.

The distribution of the okra genotypes into clusters is presented in Table 5. The dendrogram classified the 8 okra genotypes into 3 distinct clusters; cluster A, B and C comprising of 1,4 and 3 okra genotypes, respectively. Cluster A consisted of 1 okra genotype; NCB00324 showing similarity coefficient with a value of 1. Cluster B consisted of 4 okra genotypes; NCB00323, NCB00322, NCB00343 and NCB00369, which showed the similarity value of 0.875. Cluster C consisted of NCB00303, NCB00396 and NCB00466, with the similarity coefficient value of 0.750.

Table 5: Distribution of 8 Okra Genotypes into Different Clusters.

S/N	CLUSTER NUMBER	NO. OF OKRA GENOTYPES	GENOTYPES
1	A	1	NCB00324
2	B	4	NCB00323, NCB00322, NCB00343, NCB00369
3	C	3	NCB00303, NCB00396, NCB00466

The Polymorphic information content (PIC) value for each SSR markers were generally high for OKRA104, OKRA105, OKRA108 and OKRA110 50% which implies that the SSR markers were highly informative and polymorphic. This finding corroborates Kumar et al. [10], who reported high PIC among okra lines. The distribution of okra genotypes into clusters based on similarity index implies that genotypes are not closely related which implies that when selection is made for hybridization among them, the chances of obtaining good heterotic combinations as compared to those involving same clusters will be feasible. This corroborates the finding of Ahmad et al. [11] and Shinde et al., on major yield contributing characters for fruit yield, fruit length and fruit width.

Conclusion

It can be concluded from this study that the okra genotypes utilized are diverse from one another. Furthermore, it can be concluded that the 8 SSR markers utilized for this study were very informative and polymorphic. Hence, these SSR markers can be recommended for use in future Okra breeding programs. It can also be concluded that the okra genotypes are not closely related. Hence, hybridization among them promises to give good offspring.

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