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Genetic Diversity of Okra (*Abelmoschus esculentus* (L.) Moench.) Genotypes Using RAPD Primers

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Abstract

An attempt was made to assess genetic diversity among 17 cultivars of okra (*Abelmochus esculentus*) collected from diverse sources using randomly amplified polymorphic DNA (RAPD). Out of 35 primers only two could amplify such as OPB-5 and OPF-3. The primer OPB-5 amplified about 43 bands ranging from 200 to 2000 bp. Similarly OPF-3 amplified about 86 bands ranging from 200 bp to 1 kb from all the genotypes. The dendrogram developed based on Euclidean distance using Ward's method indicated 17 genotypes were grouped into two major clusters and two sub-clusters within a major cluster. Among all the genotypes studied, Arka Abhay was genetically divergent.
Key words : Genetic diversity, RAPD, Primers, Okra, Arka Abhay.

Okra (*Abelmoschus esculentus* (L.) Moench) is one of the important vegetable crops grown for its tender green fruits during spring and rainy seasons. It has high nutritive value and export potential. It is a native of Africa and belongs to the family Malvaceae. The traditional approach to characterize and evaluate is based on morphological features. Though such phenotypic evaluations are important but the data is not understood at gene level. This is because most of the economic characters are polygenically inherited and considerable interaction between genotype and environment is noticed. It is therefore essential that genetic diversity within collections be assayed in the context of total available genetic diversity for each species. Genetic engineering and biotechnology hold great potential for plant breeding as it promises to expidite the time taken to produce crop varieties with desirable characters. With the use of molecular techniques it would now be possible to hasten the transfer of desirable genes among varieties and to introduce novel genes from related wild species. Polygenic characters which were previously difficult to analyze using traditional plant breeding methods, would now be tightly tagged using molecular marker. It would also be possible to establish genetic relationships between sexually incompatible crop plants. Techniques which are particularly promising in assisting selection for desirable characters involves the molecular markers such as Randomly Amplified Polymorphic DNA's (RAPD's), Restriction Fragment Length Polymorphisms (RFLP's), micro satellites and PCR based DNA markers such as Sequence Characterized Amplified Regions (SCAR's), Sequence Tagged Sites (STS), Amplified Fragment Length Polymorphism (AFLP's) and Amplicon Length Polymorphism (ALP's) using F_2 and back cross populations, near isogenic lines, double haploids and recombinant in bread lines (1). Randomly amplified polymorphic DNA (RAPD) genetic markers are generated using polymerase chain reaction (PCR) and polymorphisms are identified by amplifying specific regions of template DNA flanked by primer sites homologous to random oligo nucleotide primers (2). Among different types of molecular markers available, RAPD's are attractive because of their simplicity, versatility, moderately ease and ability to detect relatively small amounts of variation of many crop plants (3, 4). Studies using molecular markers in okra are scanty as compared to the other major crop species, which include the reports of Martinello et al. (5) using RAPD markers and Sequence Related Amplified Polymorphism (SARP) marker by Osman et al. (6). The present investigation was conducted to assess the genetic distinctiveness and relationship among 17 genotypes using RAPD

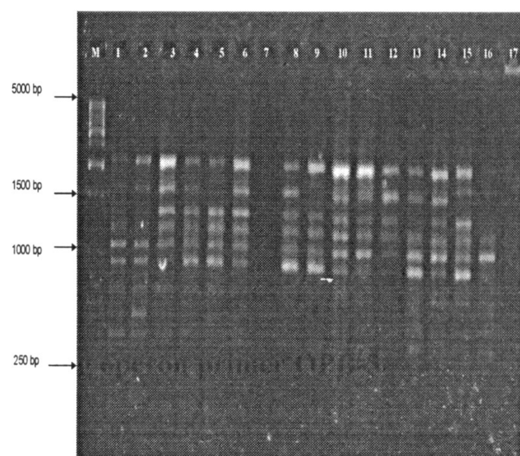

Figure 1. RAPD profiles of 17 okra accessions using operon primer OPB-5. M. Lambda marker : 1. KA-014, 2. KA-028, 3. KA-030, 4. KA-031, 5. KA-041, 6. HRB-55, 7. HRB-9-2, 8. Pusa Sawani, 9. Punjab Padmini, 10. Parbhani Kranti, 11. Arka Anamika, 12. Arka Abhay, 13. Varsha Uphar, 14. Hissar Unnat, 15. BO-13, 16. VRO-6, 17. Pusa-A4.

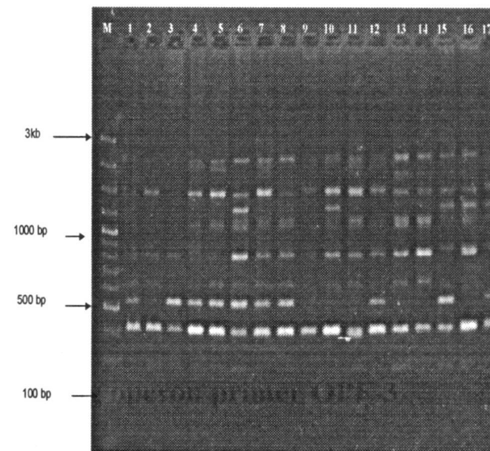

Figure 2. RAPD profiles of 17 okra accessions using operon primer OPF-3. M. Lambda marker : 1. KA-014, 2. KA-028, 3. KA-030, 4. KA-031, 5. KA-041, 6. HRB-55, 7. HRB-9-2, 8. Pusa Sawani, 9. Punjab Padmini, 10. Parbhani Kranti, 11. Arka Anamika, 12. Arka Abhay, 13. Varsha Uphar, 14.Hissar Unnat, 15. BO-13, 16. VRO-6, 17. Pusa-A4.

markers.

Methods

Seeds of 17 genotypes of okra collected from different sources were raised in pot for two weeks in the control green house and collected the young leaves from 10 to 12 days old seedlings of each genotype and dried at 45 C for 24 hours. The dried leaves were powdered by using Remi mixer for 45—60 seconds.

DNA extraction was performed using cTAB method (7) with modification. Exactly, 250 mg of the leaf powder with 20 ml extraction buffer (20 mM EDTA, 100 mM tris-base, 1.4 m NaCl, 3% cTAB and 400 mg polyvinyl pyrollidone with 1% 2-mercaptoethanol or β-morcaptoethanol) was incubated on a hot water bath at 65 C for one hour with intermittent shaking. The mixture was then cooled in an ice tray and 10 ml of cold chloroform : Isoamyl alcohol (24 : 1 vol/vol) was added and the contents were gently mixed and spun at 6,000 rpm for 20 minutes. The supernatant was transferred to a fresh tube and this step was repeated for five times till clear supernatant was obtained. To the aqueous phase, half the volume of 5M NaCl was added and gently mixed, followed by addition of 0.8 times the volume of cold propanol to precipitate DNA. The solution was kept at 4 C over night to accentuate the precipitation of DNA. The mixture was spun at 8000 rpm for 20 minutes to pellet the DNA. The pellet was washed with 70% ethanol and dried in a vacuum drier for one hour. The pellet was dissolved in 300 µl of TE buffer (10 mM Tris HCL and 1 mM EDTA) and incubated with RNAse (3 µg/ml) on a water bath at 37 C for overnight.

DNA Quantification, Amplification, Reaction Mixture

The quantity of DNA was estimated by using "Hoefers Dyna Quant" using assay buffer (10 × TNE buffer with Hoechst 33258 dye) with standard calf thymus DNA and quality was varified by gel electrophoresis on one per cent electrophoresis on agarose gel (GIBCO BRL).

The PCR procedure described by Williams et al. (2) was followed with minor modifications. PCR reaction conditions were optimized to result in informative and reproducible fingerprint profile for okra.

Amplication reaction were carried out in 25 µl reaction mixture containing template DNA (25 ng), primer (5 p mols), $MgCl_2$ (2 mM), one unit of *Taq* poly-

Table 1. Synthetic deoxyribonucleotides used as primer for amplificatiom of okra DNA. Source : Operon Technologies, Unc., Alamedia, CA 94501.

Primers	Nucleotide Sequence (5' to 3')	Number of polymorpic bands	Fragment-size
OPB-5	TGCGCCCTTC	8	200-2000
OPF-3	CCTGATCACC	9	200-1 kb

merase (Bangalore Genei and Geneitex) with 10 × buffer (1 × 50 mM KCl, 10 mM tris-HCL, 1.5 mM $MgCl_2$ and 0.1% Triton X-100) and dNTPs (10 mM each) (Finzymes). Mineral oil (one drop) was overlad to prevent the evaporation of the reaction mixture.

Amplification Conditions

Reaction mixture was performed in a thermal cycles (MJ Research, PTC 100) for 45 cycles after an initial denaturation at 95 C for two min. Each cycle consisted of one minute at 94 C (Denaturation), one minute at 35 C (Annealing) and two minutes at 72 C (extension), repeated 45 times and then with a final extension step of 5 minutes at 72 C.

Gel Electrophoresis

Agarose gel (1.2%) was prepared by using electrophoresis grade agarose. Tris borate (1 XTBE) was used as running buffer, which was also used for preparing gel. Ethidium bromide of 0.5 μg/ml of gel was added, 5 μl of PCR mixture and mixed well. Care was taken to prevent mixing of samples between the wells. A voltage of 45 was maintained for about 8—10 hours for separation of PCR amplifications and gel was photographical under UV light by using Herolab Gel Doc system (Belgium).

Data Scoring and Analysis

Fragments amplified by the primer used and molecular weights in base pairs (bp) were scored for their presence or absence (8) and a fragment was counted only if it was intense, clear and strong. Difference and or weak fragments were not scored as such fragments have been reported to possess the greatest propensity for poor reproducibility. The band sizes

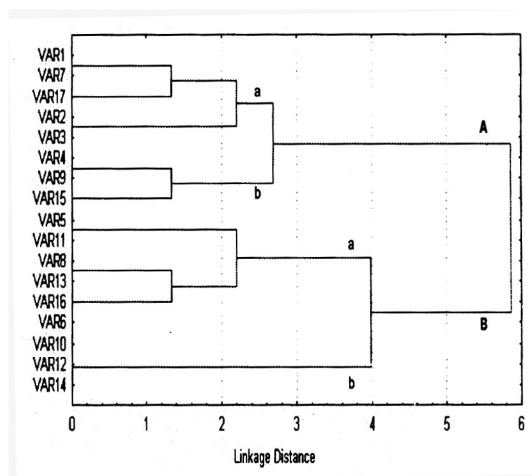

Figure 3. Tree diagram for 17 genotypes using Ward's method based on Euclidean distances. VAR1—KA-014, VAR2—KA-028, VAR3—KA-030, VAR4—KA-031, VAR5—KA-041, VAR6—HRB-55, VAR7—HRB-9-2, VAR8—Pusa Sawani, VAR9—Punjab Padmini, VAR10—Parbhani Kranti. VAR11—Arka Anamika, VAR12—Arka Abhay, VAR13—Varsha Uphar, VAR14—Hissar Unnat, VAR15—BO-13, VAR16—VRO-6, VAR17—Pusa-A4.

were estimated by using a 100 bp ladder marker, which was run along with amplified products. The binary data was subjected for cluster analysis where Ward's method and Euclidean distance are used to develop dendrogram.

Results and Discussion

The DNA recovery varied widely ranging from 48—73 μg per g of powdered leaves. DNA obtained from matured leaves was of high quality and amplifiable. Extraction of DNA from different quantities of powder (250 mg and 500 mg) with 20 ml of extraction buffer was tried with different concentrations of PVP (1, 2, 3 and 4%) and cTAB 3%. DNA isolation from 250 mg leaf powder, using 20 ml extraction buffer, 2% PVP and 3% cTAB gave good quality, high molecular weight DNA, which was confirmed by electrophoresis and spectrophotometery reading at 260/280 nm.

The PCR amplification conditions by using random primers was based on basic protocol of Welsh and Mc Clelland (9) and Williams et al. (2) with slight modifications. Amplication condition of each cycle

of PCR consisted of the following three steps, which were repeated for 45 times : Denaturation at 94 C for 1 min, annealing at 35 C for 1 min, and extension at 72 C for 2 min and final extension at 72 C for 5 min.

A concentration of 25 ng of template DNA, 1.5 mM of $MgCl_2$ and 150 μM dNTPs were found optimum for obtaining high quality amplification and intense repeatable banding patterns in okra.

Primer Selection

Out of 35 primers, only two could amplify viz., OPB-5 and OPF-3 produced 43 and 86 bands, respectively which were chosen for measuring diversity (Table 1). The results were significant and polymorphic with respect to OPB-5 and OPF-3. It was evident that the bands were 200 to 2000 bp in case of OPB-5. This primer amplified about 43 bands from all the genotypes and most of them were monomorphic in nature. The range of bands amplified was between 1—5 (Fig. 1). Similarly the primer OPF-3 amplified 86 bands ranging from 200 bp to 1kb. The number of bands produced ranged from 2 to 8. However, most of them were monomorphic in nature (Fig. 2).

Estimation of Genetic Diversity and Genetic Relatedness in Accessions of Okra

The dendrogram was constructed based on Euclidean Distance using Ward's method for all the varieties/accessions used in investigations (Fig. 3). All the 17 were grouped into two major clusters (A and B) and two sub-clusters with in a major cluster (a and b). There was 5.9 units of genetic distance between cluster A and B. Within cluster A, there was 2.6 units of genetic distance between cluster a (KA-O28, Pusa A4 and HRB-9-2) and b (BO-13 and Punjab Padmini) and in cluster B, there was 4.0 units of genetic distance between sub cluster a (VRO-6, Varsha Upahar, and Arks Anamika) and sub-cluster b (Arka Abhay). Arka Abhay showed maximum genetic divergence (4.0 and 5.9) units of genetic distance for cluster B and A respectively. The remaining genotypes were not included into these clusters because if low polymorphism.

There was a considerable variation existing among genotypes and Arka Abhay was genetically divergent. The existence of diversity might be due to intrinsic genomic features. Arka Abhay though was tolerant to fruit borer damage and no specific bands were associated. The most effectiveness of RAPD primers in differentiating of genetic divergence of okra cultivars was also established by several other workers (5, 6, 10). In this investigation Arka Abhay can be used as a male parent to create wider variability for fruit borer resistance.

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