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**Research Article**

# Assessment of genetic relatedness among okra genotypes [*abelmoschus esculentus* (l.) Moench] using rapd markers

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**Abstract:**
DNA based RAPD (Randomly Amplification of Polymorphic DNA) markers have been used extensively to study genetic relationships in number of crop plants. In this study, 44 okra genotypes collected from different parts of India, were selected to assess genetic distinctiveness and relatedness. Total genomic DNA was extracted and subjected to RAPD analysis using 14 arbitrary 10 mer primers. The molecular analysis showed that all the fourteen primers used revealed clear distinction between the genotypes and they generated a total of 104 RAPD bands most of which were polymorphic across accessions (74.03%). The number of bands resolved per amplification was primer dependent and varied from 4 (OPV-07, OPV-08) to 11 (OPD-05) with average number of bands per primer was 7.41. RAPD data were used to calculate a Squared Euclidean Distance matrix, and based on this, cluster analysis was done using minimum variance algorithm. Cluster analysis showed two major groups. Each sub-group was characterized using morphological and genetic characteristics of the respective genotypes.

**Key words:** *Abelmoschus esculentus* (L.) Moench, Genetic diversity, RAPD

## Introduction

Okra is an important vegetable crop in India, West Africa, South-East, Asia, U.S.A, Brazil, Australia and Turkey. In some regions, the leaves are also used for human consumption. The value of a germplasm collection depends not only on the number of accessions it contains, but also upon the diversity present in those accessions (Ren *et al.,* 1995). Knowledge of genetic diversity and relationships among okra germplasm may play significant role in breeding programmes to biotic and abiotic stresses as it helps to screening of desired genotypes for our trait of interest. Ariyo, 1993 reported that the within species of *Ablelmoschus esculentus,* variation found among the 30 African genotypes was considerably high based on phenotypic assessment.

Characterization and quantification of genetic diversity has long been a major goal in evolutionary biology and various genetic improvement programs. Information on the genetic diversity within and among closely related crop varieties is essential for a rational use of plant genetic resources. Diversity based on morphological characters usually varies with environments and evaluation of traits requires growing the plants to full maturity prior to identification of diverse genotypes. Now, the rapid development of biotechnology allows easy analysis of large number of loci distributed throughout the genome of the plants. Molecular markers have proven to be powerful tools in the assessment of genetic variation and in elucidation of genetic relationships within and among species (Chakravarthi and Naravaneni, 2006). To conserve and use these plant genetic resources effectively, it is essential to develop markers that not only distinguish individuals and accessions, but also reflect the inherent diversity and relationships among collection holdings (Kresovich and McFerson, 1992).

Random amplified polymorphism DNA (RAPD) markers have been used to characterize identities and relationships of various crops (Tingey and Deltufo, 1993; Lima *et al*., 2002). Kresovich *et al*. (1992) showed that these markers could be of great value in genetic resources management as a quick, costeffective and reliable method for identification, measurement of variation, and determination of similarity at the intra-specific level.

Martinello *et al*. (2001) demonstrated that genetic distance based on molecular data (RAPD), average distance from the morphological data and descriptors generated by quantitative data has similar dendrograms pattern in *Abelmoschus spp*. Unfortunately, most of the tropical vegetables such as okra lacked detailed data at biochemical level (Hamon and Noirot 1991). The only study reported is by Martinello *et al*. (2001) using Randomly Amplified polymorphic DNA (RAPD) marker and Sequence Related Amplified polymorphism (SRAP) marker by Gulsen *et al.,* (2007). The information based on DNA marker would be of great interest in okra breeding programme data to determine whether phenotypically similar cultivars are genetically similar or not (Duzyaman, 2005). The present study aimed to estimate genetic relatedness among a set of 44 okra genotypes cultivated in different parts of the country using RAPD markers.

## Material and methods

The experiments were carried out in the Molecular Biology Lab, Department of Plant Biotechnology, Indian Institute of Horticultural Research (IIHR), Bangalore during 2009-2010.

### Plant material

Forty-four diverse okra genotypes collected from different parts of India were used for this study (Table 1). Seeds were raised in plastic pot filled with soil for two weeks in the green house at an average minimum and maximum temperature of $24^0$C and $31^0$C respectively. Young, healthy third to fourth leaf from the bottom of the okra genotypes were collected from the field in an aluminium foil and washed thoroughly with distilled water and air dried to remove moisture. 100-110 mg of leaf material was used to extract DNA.

### DNA Extraction

The isolation of genomic DNA from okra was carried out using the CTAB mini-prep method. 100-110 mg of leaf tissues were ground to fine powder using liquid nitrogen. A pinch of PVPP and sodium metabisulphate were added to this and transferred to 1ml of extraction buffer (containing 2 % of w/v CTAB, 1.4 M NaCl, 20 mM EDTA, 0.1 % β-mercaptoethanol, 100 mM Tris pH 8.0) preheated to $60^o$C for 1 hr with occasional shaking. The homogenate was cooled to room temperature and extracted with 5 ml of chloroform: amyl alcohol (24:1) centrifuged at 10,000 rpm for 10 minutes and the clear aqueous phase separated. To this equal volume of potassium acetate mixture (2.5 mM) and chloroform: amyl alcohol mixture added, centrifuged at 10,000 rpm for 10 minutes and the clear aqueous phase separated and added equal volume of cold isoproponol and stored at $-20^o$C for one hour. Then the contents were centrifuged at 10,000 rpm for five minutes and the clear aqueous phase separated and then added 1 ml of absolute ethanol (99.9 %) and stored at $-20^o$C for one hour. This was again centrifuged at 10000 rpm for 5 minutes and the supernatant decanted retaining the pellet. The pellet was washed twice with 76 % v/v ethanol and then vacuum dried. The pellet was then dissolved in 50 µl of Tris-EDTA buffer of pH 8.0 containing RNAase (10 µg/ ml) and incubated at $37^0$C for 2hr to overcome the problem with RNA contaminations and then the DNA concentrations were quantified through 260 nm spectrophotometer.

### RAPD analysis

DNA amplification was done using 14 arbitrary decamer primers OPA, D, V and X (Table 2) following Williams *et al*. (1990). PCR reactions were carried out in a volume of 25 µl containing $H_2O$ (6.67 µl) 10 X Reaction buffers (2.5 µl), 1mM dNTPs (4.0 µl), 3pM Primer (5.0 µl), 2.5 mM $MgCl_2$ (2.5 µl), 20 ng Template DNA (4.0 µl) and 1U/ µl *Taq* DNA polymerase (0.33 µl). Amplification was performed in a programmable thermo cycler at $94^0$C for 3 min; 2 cycles of $94^0$C for 1 min, $40^0$ for 1 min and $72^0$C for 2 min; and 39 cycles of $94^0$C for 30 sec, $40^0$C for 30 sec and $72^0$C for 1min and extension at $72^0$C for 2 min. Amplification products were separated on 1.5% agarose gel containing 0.5 µg ethidium bromide per ml. Electrophoresis was carried out 5 v/cm for 2.5 hr. The gels were viewed and photographed under UV transilluminator.

### Statistical analysis

The banding pattern from RAPD analysis for each primer was scored by visual observation. The presence of an amplification product (band) in each position was recorded as 1 and absence as 0. Based on presence/absence, a Squared Euclidean Distance matrix was calculated to estimate all pair-wise differences in the amplification product for all genotypes (Sokal and Sneath, 1973). Based on the distance matrix cluster analysis was done using minimum variance algorithm (Ward, 1963). Further, Principle Component Analysis (PCA) was done to estimate relationship among genotypes and dendrograms was constructed with the help of computer package STATISTICA.

## Results and discussions

In any plant breeding programme, assessment of parental divergence is an important and foremost objective. The threat to genetic erosion has led to a significant interest in the assessment of genetic diversity in germplasm collections (Manifesto *et al*., 2001). It helps in identifying the desirable parents for hybridization programme. Molecular markers are useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects and allow cultivar identification early in plant development. The molecular markers based on differences in DNA sequences between individuals generally detect more polymorphisms than morphological and protein based markers (Mignouna *et al*., 1998).

### Polymorphism of RAPD markers

The RAPD analysis of 44 okra genotypes was done using 14 arbitrary decamer primers. All 14 primers gave good amplification with polymorphisms and they were generated a total of 104 RAPD bands most of which were polymorphic across accessions (74.03%) because they were able to differentiate at least any two of the 44 okra genotypes at a time (Table 2). The number of bands per primer varied from 4 (OPV-07, OPV-08) to 11 (OPD-05) with an average number of 7.41 bands per primer. The size range of amplification products also differed with selected primers as well as the genotypes and ranged from 250bp to 1500bp (Figure 1). Using RAPD, Martinello *et al*. (2001**)** identified 103 amplification products in okra with 31 primers. One of the advantages of the RAPD method is that the arbitrarily designed primers can potentially anneal to homologous sequences in the entire genome, providing greater opportunities to uncover regions (Williams *et al*., 1990). The RAPD profiles obtained by using various primers varied from one another. This may be due to the difference in primer sequences. The different primers were capable of developing different banding patterns. Out of 14, the primer OPD-05 produced maximum number (11) of total bands of which 10 bands were (90.90%) polymorphic.

The binary data was utilized for cluster and principle component analysis (PCA). The genetic dissimilarity value in the distance matrix ranged from 0 to 47, suggesting a narrow genetic base within the okra genotypes used in the study. The highest genetic distance of 47 was observed between IIHR-219 & IIHR-231, indicating that these are genetically more distinct. The primary reason for narrow genetic base in okra genotypes is the often-cross pollination nature.

### Cluster Analysis

The cluster analysis and PCA clearly showed two major groups of okra genotypes (Figure 2and 3). In PCA analysis the first two components accounted for 42% variation. It is clearly evident from the dendrogram (Figure 2) that, all the 44 okra accessions are grouped into two major clusters namely A and B. Total dissimilarity value across all the accession was 81 units. Cluster A differed from cluster B with 62 units. Cluster A comprises a total of three genotypes of which IIHR-15, IIHR-18 of same geographical locations and IIHR-231. The cluster B comprises a total of 41 genotypes. Further, cluster B subdivides into 11 sub clusters namely $B_1$ to $B_{11}$. A phylogenetic tree constructed on the basis of dissimilarity to 35 units between the genotypes made it possible to classify 44 okra genotypes into a total of 12 distinct clusters.

Sub cluster $B_1$ consisted of three genotypes namely IIHR-20, IIHR-31 and IIHR-249. The sub cluster $B_2$ comprises IIHR-251 A and IIHR-252 genotypes of same species i.e. *Abelmoschus callei* which is grouped with IIHR-10 and IIHR-108 genotypes of *A. esculentus* species. This is consistent with the results obtained from Martinello *et al*. (1996). Sub cluster $B_3$ included three genotypes IIHR-55, IIHR-101 and IIHR-239. Sub cluster $B_4$ had six genotypes IIHR-04, IIHR-134, IIHR-133, IIHR-242, IIHR-240 and IIHR-241. Similarly the sub cluster $B_5$ consisted of six genotypes as IIHR-72, IIHR-225, IIHR-81, IIHR-91, IIHR-116 and IIHR-245. Sub cluster $B_6$ contained four genotypes namely IIHR-230, IIHR-244, IIHR-243 and IIHR-248. Sub cluster $B_7$ comprised only with two genotypes namely IIHR-246 and IIHR-247. Sub cluster $B_8$ consisted with of five genotypes namely IIHR-181, IIHR-219, IIHR-226, IIHR-182 and IIHR-213. Sub cluster $B_9$ comprised with two genotypes namely IIHR-237 and IHR-238. Finally the sub cluster $B_{10}$ comprised with five genotypes namely IIHR-224, IIHR-227, IIHR-229, IIHR-232 & IIHR-238 and the sub cluster $B_{11}$ involved with only one genotype called IIHR-250A. The dissimilarity between $B_1$ and $B_2$ sub cluster was 26 units and similarly $B_3$ and $B_4$ was 32 units, $B_5$ and $B_6$ was 32.5 units, $B_7$ was 45 units, $B_8$ and $B_9$ was 30.5 units and finally 33 units were dissimilar of the sub cluster $B_{10}$. However, most of the clusters did not associate with geographic origins of okra genotypes.

From the present study, it is concluded that, the maximum diversity expressed between the genotypes

IIHR-219 & IIHR-231 (47%), IIHR-231 & IIHR-243 (45%) followed by IIHR-226 & IIHR-231, IIHR-230 & IIHR-231, IIHR-231 & IIHR-244, IIHR-231 & IIHR-248 and IIHR-231 & IIHR-251A (44%) may be exploited by effective crossing between these genotypes to obtain desirable segregates for further selection of superior lines in early stages of crop growth itself by exploiting the genetic distance from molecular marker data which helps to identify genotypes for mapping populations and also to identify molecular markers linked to desirable traits (resistance to YVMV) by marker assisted selection (MAS).

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**Table 1: List of the genotypes used for experiments**

| Accession number | Donor's name | Taxonomic name | Sources of collection |
|---|---|---|---|
| IIHR 15 | Lam Hybrid 1 | *Abelmoschus esculentus* | LAM, Hyderabad |
| IIHR 18 | Lam Hybrid 2 | *A. esculentus* | LAM, Hyderabad |
| IIHR 20 | IIHR 20-31 | *A. esculentus* | IIHR, Bangalore, KA |
| IIHR 31 | Philippines Long | *A. esculentus* | NBPGR, New Delhi |
| IIHR 55 | GOH 4 | *A. esculentus* | GAU, Gujarat |
| IIHR 72 | Clemson Spineless | *A. esculentus* | Bentley seeds, New York, USA |
| IIHR 81 | SA 2605 | *A. esculentus* | FSI coastal region, USA |
| IIHR 91 | HRB 92 | *A. esculentus* | HAU, Hissar |
| IIHR 101 | Pure Line | *A. esculentus* | NBPGR, New Delhi |
| IIHR 108 | Pure Line | *A. esculentus* | NBPGR, New Delhi |
| IIHR 10 | Inter specific hybrid | *A. esculentus* | IIHR, Bangalore, KA |
| IIHR 116 | Pure Line | *A. esculentus* | NBPGR, New Delhi |
| IIHR 04 | Inter specific hybrid | *A. esculentus* | IIHR, Bangalore, KA |
| IIHR 133 | Pure Line | *A. esculentus* | NBPGR, New Delhi |
| IIHR 134 | Pure Line | *A. esculentus* | NBPGR, New Delhi |
| IIHR 181 | Gopalapur Local | *A. esculentus* | NBPGR, New Delhi |
| IIHR 182 | Nalanda Local | *A. esculentus* | NBPGR, New Delhi |
| IIHR 213 | Varanasi Ridges | *A. esculentus* | Farmers field, Varanasi, UP |
| IIHR 219 | Ramanagaram Local | *A. esculentus* | Farmers field, Mysore, KA |
| IIHR 224 | Puttur 2 | *A. esculentus* | Farmers field, DKKarnataka |
| IIHR 225 | Puttur 3 | *A. esculentus* | Farmers field, DKKarnataka |
| IIHR 226 | Kodippay 1 | *A. esculentus* | Farmers field, DK Karnataka |
| IIHR 227 | Sadarapalli 1 | *A. esculentus* | Farmers field, DK Karnataka |
| IIHR 229 | Hejamady 1 | *A. esculentus* | Farmers field, Udupi, KA |
| IIHR 230 | Panduneelavar1 | *A. esculentus* | Farmers field, Udupi, KA |
| IIHR 231 | Panduneelavar2 | *A. esculentus* | Farmers field, Udupi, KA |
| IIHR 232 | Kokkaranai | *A. esculentus* | Farmers field, Udupi, KA |
| IIHR 233 | Nigerian Collection | *A. esculentus* | Nigeria |
| IIHR 237 | PN 10 | *A. esculentus* | Farmers field, India |
| IIHR 238 | PN 11 | *A. esculentus* | Andra Pradesh, India |
| IIHR 239 | PN 12 | *A. esculentus* | Farmers field, India |
| IIHR 240 | PN 13 | *A. esculentus* | Andra Pradesh, India |
| IIHR 241 | VRO 6 | *A. esculentus* | IIVR, Varanasi |
| IIHR 242 | JNDO 5 | *A. esculentus* | Junagharh, Gujarat |
| IIHR 243 | JR-04-92 | *A. esculentus* | Farmers field, India |
| IIHR 244 | JSR-04-101 | *A. esculentus* | Farmers field, India |
| IIHR 245 | JSR-04-125 | *A. esculentus* | Farmers field, India |
| IIHR 246 | JSR-04-133 | *A. esculentus* | Farmers field, India |
| IIHR 247 | JSR-04-134 | *A. esculentus* | Farmers field, India |
| IIHR 248 | JR-04-41 | *A. esculentus* | Farmers field, Mandya, KA |
| IIHR 249 | JR-04-48 | *A. esculentus* | Farmers field, Shimoga, KA |
| IIHR 250A | JR-04-50 | *A. esculentus* | Farmers field, Shimoga, KA |
| IIHR 251A | JR-04-73 | *Abelmoscus callei* | Farmers field, Goa |
| IIHR 252 | VRO 5 | *A. callei* | IIVR, Varanasi |

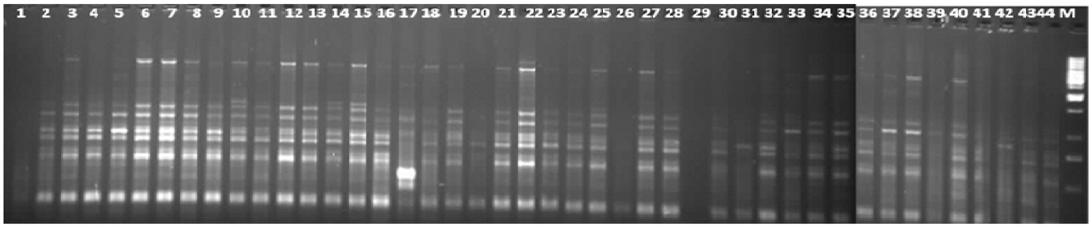

**Figure 1: RAPD profiles of 44 okra genotypes using primer OPD-05 (Lane M- 1 kb DNA ladder, Lane 1-44 represent the genotypes in the same order as listed in Table 1**

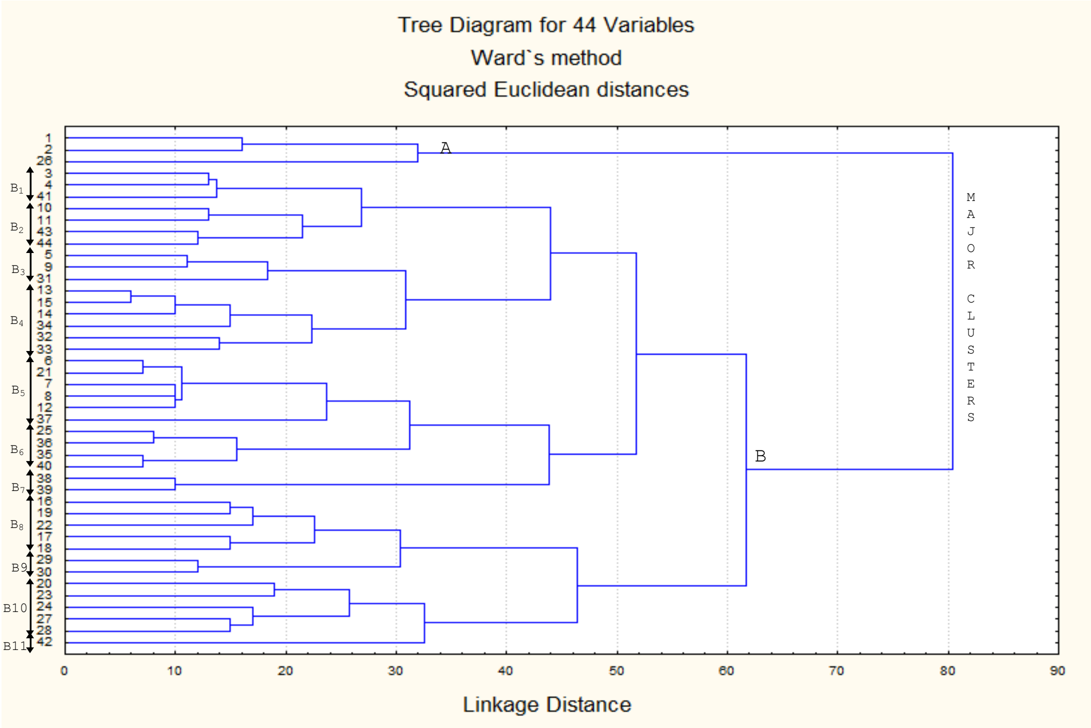

**Fig 2: Dendrogram showing the genetic diversity among 44 okra accessions using cluster analysis of RAPD data**(*for* 1 to 44 accessions *ref. Table 1*)

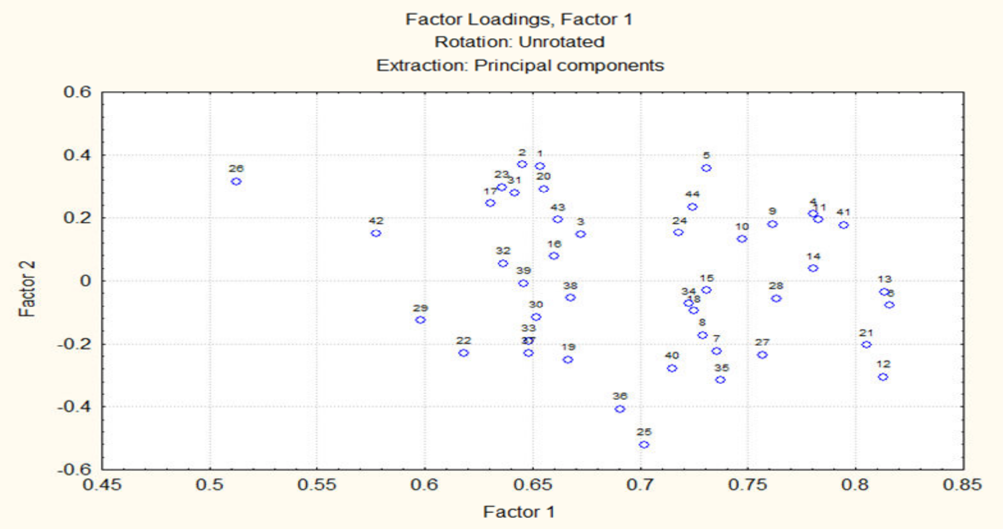

**Fig 3: Genetic diversity among 44 okra accessions using (2-dimensional space) principal component analysis (PCA) of RAPD data** (*for* 1 to 44 accessions *ref. Table 1*)

**Table 2: Polymorphism in 44 okra genotypes generated by 14 RAPD primers**

| Primer | Primer sequence (5' to 3') | No. of bands produced | No. of polymorphic bands | Percentage of polymorphism (%) |
|---|---|---|---|---|
| OPA 02 | TGCCGAGCTG | 6 | 5 | 83.33 |
| OPD 03 | GTCGCCGTCA | 7 | 5 | 71.42 |
| OPD 05 | TGAGCGGACA | 11 | 10 | 90.90 |
| OPT 01 | GGGCCACTCA | 9 | 5 | 55.55 |
| OPT 02 | GGAGAGACTC | 7 | 4 | 80.00 |
| OPT 04 | CACAGAGGGA | 8 | 6 | 75.00 |
| OPV 02 | AGTCACTCCC | 10 | 8 | 80.00 |
| OPV 03 | CTCCCTGCAA | 7 | 5 | 71.42 |
| OPV 04 | CCCCTCACGA | 8 | 6 | 75.00 |
| OPV 05 | TCCGAGAGGG | 7 | 5 | 71.42 |
| OPV 06 | ACGCCCAGGT | 9 | 7 | 77.77 |
| OPV 07 | GAAGCCAGCC | 4 | 3 | 75.00 |
| OPV 08 | GGACGGCGTT | 4 | 3 | 75.00 |
| OPX 17 | GACACGGACC | 7 | 5 | 71.42 |
| | **Mean** | **7.42** | **5.5** | **74.12** |
| | **Total** | **104** | **77** | **74.03** |