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MOLECULAR CHARACTERIZATION AND DIVERSITY ANALYSIS OF *ABELMOSCHUS ESCULENTUS* GERMPLASM

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Summary

Twenty-two genotypes of *Abelmoschus esculentus L. Moench,* including resistant varieties, hybrid varieties and susceptible varieties for YVMV infection, were collected and analyzed for genetic diversity. 20 random primers were used of which 15 resulted in polymorphic, scorable and reproducible. The UPGMA based dendogram grouped 22 genotypes into three major clusters with one genotype placed independently at one end of the dendogram. The study shows the separation of individuals on the basis of their response towards YVMV infection with some exceptions. The present study reveals that PCR based fingerprinting techniques as RAPD is effective, promising and informative for estimating the extent of genetic diversity as well as to determine the pattern of genetic relationships between different genotypes of *A. esculentus*.

सारांश

प्रस्तुत शोध में भिण्डी की 22 प्रजातियों जिसमें YVMV के लिए प्रतिरोधक, संकर एवं संवेदनशील शामिल है, की अनुवांशिक विविधता का अध्यन किया गया है। इस कार्य के लिए 20 RAPD मारकर का प्रयोग किया गया जिसमें से 15 ने बहुरूपी विश्लेषण करने योग्य एवं बार–बार एक समान परिणाम दिये। UPGMA पर आधारित डैन्डोग्राम ने 22 प्रजातियों को 3 मुख्य समूह में बाँटा। परिणाम दर्शाते हैं कि प्रजातियाँ YVMV के समक्ष अपने प्रतिरोधन के अनुसार अलग–अलग समूह में एकत्र हुयी। प्रस्तुत शोध व्यक्त करता है कि PCR पर आधारित RAPD तकनीक भिण्डी में अनुवांशिक विविधता जानने के लिए एवं अनुवांशिक सम्बन्ध जानने के लिए आशाजनक एवं सूचनादायक हैं।

Introduction

Information on the genetic diversity within among closely related crop varieties is essential for a rational use of plant genetic resources. There are different ways to assess genetic diversity and genetic structure within a species. 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). RAPD (Williams *et al*., 1990), a PCR based technique has resolved most of the technical obstacles owing to its cost effective and easy to perform approach (Singh *et al*., 1999). RAPD has therefore been extensively used in assessing genetic relationship amongst various accessions of different plant species (Deshwal *et al*., 2005; Monteleone *et al*., 2006) and for many other purposes.

Okra (*Abelmoschus esculentus* L. Moench), is an economically important vegetable crop of family Malvaceae, which is grown in tropical and sub-tropical parts of the world. Okra (commonly known as lady finger, bhindi, Gumbo etc.), provides an important source of vitamins, calcium, potassium and other minerals matters. Studies using molecular markers in okra (Martinello *et al*., 2001; Gulsen *et al*., 2007). Knowledge of genetic diversity and relationship among okra germplasm based on DNA marker may play significant role in breeding programmes of okra.

Materials and Methods

Twenty two okra varieties of *Abelmoschus esuclentus* (Figure-1) were collected from the HRC, Sardar Vallabhbhai Patel University of Agriculture And Technology. All varieties were grown in pots with 5-5 seed of two varieties in a single pot for collection of leaf tissue. The data of seed germination and time taken for germination was recorded. After germination of seed, leaf samples of each variety were collected and wrapped in Aluminum foil. The packed leaf tissue were then stored in (-80^{0}c) deep freezer.

DNA isolation: The total genomic DNA was isolated from various leaf samples following the method of Doyle and Doyle (1990) as modified by Vaishali et al (2008), with an additional step of purification as follows. The isolated DNA was checked on 0.8% agarose (Sambrook *et al*., 1989). The gel was visualized under Mini-Transilluminator (Bio Rad, India). The gel was then photographed and analyzed using Lamda

DNA double digest (Bangalore Genei Pvt. Ltd.) as a standard.

RAPD profiling: A total of 20 random decamer primers (custom synthesized by Bangalore Genei Pvt. Ltd., GC content >50%) were used for RAPD analysis. DNA amplification reaction was performed in 25 μl reaction volume which contained an end concentration 2.5 mM each of the dNTPs, 1 U/μl Taq polymerase enzyme, 25 ng DNA template and 10 ng primer in Taq polymerase assay buffer (1 X) (10 X buffer contains 100 mM Tris-Cl, 500 mM KCl, 15 mM $MgCl_2$ and 0.1 % gelatin). Amplification reaction was carried out in a Eppendrof Thermal cycler with the following thermal profile: one cycle of 4 min at 94ºC (initial denaturation) followed by 45 cycles of 15 sec at 94ºC (denaturation), 45 sec at 40ºC (primer annealing) and 90 sec at 72ºC (primer elongation), and finally one cycle of 4 min at 72ºC (final extension). Amplified PCR products were separated on 1.5 % (w/v) agarose gel in 1 X TBE (10 X TBE buffer contained 108 g tris base, 9.3 g EDTA and 55 g boric acid in 1 litre double distilled water, pH 8.3). Electrophoresis was performed at 100 volt for 2 hr. Then the gel was visualized, photographed and analysed using low range DNA ruler (Bangalore Genei Pvt. Ltd.) as a molecular size marker. The reproducibility of the amplification products was checked twice for each polymorphic primer.

Data analysis: RAPD bands were scored for presence (1) and absence (0) across all *okra* accessions for each primer. The pair wise genetic similarities among all pairs of samples were estimated with Jaccard's coefficient (Jaccard, 1908). The statistical analysis was carried out by using NTSYS-PC software (version 2.11s) (Rohlf 2000). In order to group genotypes into discrete clusters a dendrogram was constructed by employing UPGMA (Sneath and Sokal, 1973). Resolving power (Rp) and gene diversity for each primer was calculated following Prevost and Wilkinson's (1999) as described by (Vaishali *et al*., 2008).

Results and Discussion

Out of 20 random primers used for RAPD profiling, 18 primers gave amplification (Figure 1). OF them 15 primers gave scorable and reproducible results, hence considered for genetic diversity analysis (Table 1). The polymorphic primes generate a total of 56 amplification fragments with 96.4 % polymorphism. The number of markers detected by each primer depends on prime

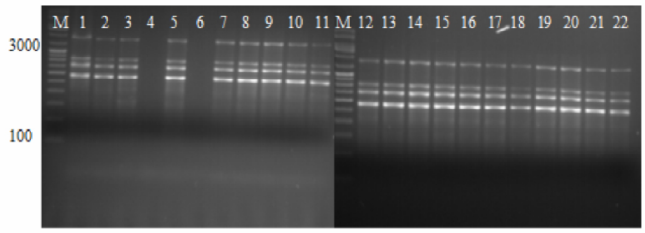

Figure 1. RAPD profile of *Abelmoschus esculentus* germplasm with primer NJ-3(A), NJ-9 (B)

Table 1. Polymorphic primers with their corresponding gene diversity and resolving power

Primer code	Sequence	Tm	Total no of Bands	Polymorphic bands	Gene diversity	Resolving power
NJ-1	AAATCGGAGC	30	5	5	0.681	2.00
NJ-2	GTCCTACTCG	32	4	4	0.419	0.83
NJ-3	GTCCTTAGCG	32	6	6	0.705	3.18
NJ-4	TGCGCGATCG	34	3	3	0.374	0.64
NJ-5	AACGTACGCG	32	6	6	0.792	5.45
NJ-6	GCACGCCGGA	36	7	7	0.593	3.91
NJ-7	CACCCTGCGC	36	1	1	0.702	0.91
NJ-8	CTATCGCCGC	34	2	1	0.235	0.54
NJ-9	CGGGATCCGC	36	4	4	0.173	0.73
NJ-10	GCGAATTCCG	32	5	5	0.702	1.27
NJ-11	CCCTGCAGGC	36	5	5	0.530	2.65
NJ-12	CCAAGCTTGC	32	2	2	0.173	0.36
NJ-13	GTGCAATGAG	30	2	1	0.235	0.55
NJ-14	AGGATACGTG	30	1	1	0.702	0.91
NJ-15	AAGATAGCGC	30	3	3	0.374	0.64

sequence and the extent of variation is genotype specific (Upadhyaya *et al*., 2004). This is understandable as product amplified depends upon the sequence of random primers and their compatibility within genomic DNA. The number of markers detected by each primer depends on primer sequence and the extent of genetic variation, which is genotype specific (Upadhyay *et al*., 2004).

Gene diversity was calculated for each primer, which varied from 0.17-0.79 values with a mean diversity of 0.49. Resolving power (Rp) for each primer was calculated following Prevost and Wilkinson's (1999) method and found to be varied from a lower value of 0.36 to a higher value of 5.45. On the basis of resolving power the primer NJ-5 found to be more informative. Resolving power has been found to correlate strongly with genotype diagnosis and so has potential for a number of applications. Nevertheless, these could be ranked according to their Rp values under the reasonable premise that primers with higher Rp value have a greater capacity to separate different accessions (Prevost and Wilkinson, 1999).

Genetic similarity matrix and cluster analysis**:** RAPD data were used to make pair wise comparison of the genotypes based on shared and unique products to

generate a similarity matrix with NTSYS-PC (version 2.11s). Genetic relationships among genotypes were evaluated by generating a similarity matrix based on Jaccard's coefficient, ranging from 0.29 to 0.98. The maximum similarity value showed by KS-446 and KS-447 (coefficient value 0.98). Both of them were susceptible varieties. The minimum similarity was showed by the genotypes from CKS-311 and AB-2 with a coefficient value 0.28. The low value of similarity coefficient may be due to the fact that the varieties were succeptible and resistant respectively.

The similarity matrix representing Jaccard's coefficient was used to cluster the data following the UPGMA algorithm. The resultant phenogram grouped 22 genotypess into three major clusters with one genotype placed independently at one end of the dendrogram (Figure 2). The cluster-I grouped ten genotypes into two subclusters. The subcluster I grouped only two genotypes which were succeptible. The subcluster II grouped 8 genotypes including two hybrid variety and two resistant varieties. The cluster-II was represented by six genotypes. The major cluster is subdivided into two subclusters with three genotypes in each subcluster. All the members of this major cluster were succeptible. Cluster-III grouped five genotypes viz K.S-423, 437, 427, 446, 305. All of them were succeptible varieties. Besides these, one genotype ie. ARKA did not grouped with any genotype and placed at one end of the dendrogram. Cluster analysis shows the separation of individuals on the basis of their response towards YVMV infection with some exceptions. The partitioning and distribution of variability complex may be cited as reason for the grouping of samples in different clusters.

The present study reveals that PCR based fingerprinting techniques as RAPD is effective, promising and

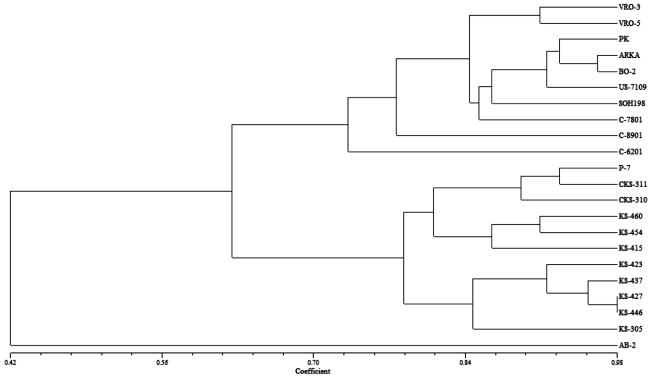

Figure 2. Dendogram of 22 genotypes of *Abelmoschus esculentus* based on RAPD

informative for estimating the extent of genetic diversity as well as to determine the pattern of genetic relationships between different genotypes of *A. esculentus*, with polymorphism levels sufficient to establish informative fingerprints with relatively fewer primer sets. The informative primers identified in our studies will be useful in genetic analysis of *A. esculentus* accessions in germplasm holdings. Information on genetic relatedness among genetic resources of crop plants is useful not for breeding purposed but also for the conservation of germplasm.

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