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ASSESSMENT OF GENETIC DIVERSITY IN DIVERSE GENOTYPES OF EGG PLANT (*SOLANUM MELONGENA* L.) USING RAPD MARKERS

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ABSTRACT
The present study was aim to analyze genetic diversity among diverse genotypes egg plant using Random Amplified Polymorphic DNA (RAPD) technique. Twenty RAPD primers were used to amplify genomic DNA from 24 brinjal genotypes. Out of 20 primers, of which only 5 primers (6.5%) showed polymorphism and remaining 15 showed monomorphism. The DNA fragments of various sizes ranging from 500 bp to 1300 bp were amplified in various egg plant genotypes. Estimates of genetic distance (GD) ranged from 0.27 to 1.05. Cluster analysis showed that genotypes were clustered in two groups A and B on the basis of distinct and significant genotypes. The UPGMA dendrogram based on genetic distance segregated the 24 genotypes fall into six clusters. Thus the genotypes included in the diverse clusters could be used as a parent for hybridization in order to obtain a high heterotic response and better segregates in eggplant. Therefore, identification of genetically distinct varieties using RAPD marker could be a potential tool for Eggplant improvement.

INTRODUCTION

Eggplant (*Solanum melongena* L., 2n=24) is one of the most important vegetable crop in Central, Southern, Southern-Eastern Asia, and African countries (Kalloo, 1993). It is the third most versatile crop, grown throughout the world and belongs to the genus *Solanum*, of the Solanaceae family (Knapp *et al.*, 2004). In India it is cultivated throughout the country including tropical, subtropical and temperate regions. India is the second largest producer (11.89 million tons) of world total production, which is estimated of 43.17 million tons (FAO Data, 2012, NHB, 2011). It is a valuable member of human diet and also used for the treatment of diabetes, bronchitis, asthma, dysuria, dysentery etc. diseases in Asia, especially in India, which is primarily diversity centre of the species (Daunay *et al.*, 2001; Kashyap *et al.*, 2003).

Information regarding genetic diversity and genetic relationships among different genotypes is very valuable in crop improvement. Identification, naming and classification of a species are including in systemic which define diversity and generally based on morphological data. High level of morphological diversity at the level of genera, species and cultivars creates confusion about its diversity (Daunay *et al.*, 1991; Weising *et al.* 1995; Doganlar *et al.*, 2002). The confusion in define diversity/taxonomy affinity of eggplant is complex due to the fact that phylogenetic relationships established among systemic (define to diversity) are mainly based on morphological features (Karihaloo *et al.*, 1995; Kumar *et al.*, 2013) cross ability and F1 fertility (Baksh, 1979; Hasan and Lester, 1990; Lester and Hassan, 1991; Furini and Wunder, 2004). These parameters are, however, insufficient to establish genetic affinities, because egg plant can be crossed not only to putative progenitors but also to more distantly related species (Daunay *et al.*, 1991). Moreover, because of the existence of a high level of morphological variability, morphological data can lead to ambiguous interpretations. Morphological traits have certain limitations such as difficulty in scoring homozygous from heterozygous individuals, influence of environment in equating phenotypes with genotypes, less availability of scorable markers and some traits expressed at letter stage of the development etc. Morphologically it shows polymorphism but confirmation is done through molecular analysis provide a clear-cut outcome. On the other hand molecular markers have many advantages such as abundance in polymorphism, can be observed at any growth stage, no pleiotropic effect, less affected by environment and subjected to rapid, accurate and simple detection (Singh *et al.*, 2006). Since molecular markers reveals difference at DNA level, therefore, represent an extremely powerful tool for assessment of genetic diversity among elite breeding lines, populations and also in wild species with high precision. Among molecular markers, RAPD markers became popular and attractive for automated breeding applications because of its simplicity, non involvement of radioactivity and ease of use in well equipped laboratory, random nature of

binding of the primers, thereby screening a much larger portion of the organelle genome diversity, requires only small amount of DNA (10-25 ng), can be performed in few hours. Thus, due to importance of the egg plant in national economy as well as international market the crop improvement is essential. In plant breeding programme for crop improvement first step is selection of genetically diverse genotypes. Therefore, objective of the present investigation was employed for genetic diversity analysis in a set of 24 egg plant genotypes using Random Amplified Polymorphic DNA (RAPD) markers to utilize in the breeding programme for genotypes improvement.

MATERIALS AND METHODS

Plant material

In the present study 24 diverse egg plant genotypes were procured from germplasm collection of egg plant, division of crop improvement and plant genetic resources, Indian Institute of Vegetable Research (IIVR), Varanasi mentioned in Table 1. The crop was raised in randomized block design at the research farm of IIVR, Varanasi during 2010. All the morphological traits were recorded for analysis of variance.

Genomic DNA isolation

The laboratory experiment was conducted in the Molecular Biology Laboratory, Indian Institute of Vegetable Research, Varanasi during 2011. Total genomic DNA was isolated from the three week old leaves according method described by Doyle and Doyle [20] (1987) with minor modification. Leaves were ground in liquid nitrogen using mortar and pestle to fine powder. The grind leaf powder was transferred into a 1.5 ml centrifuge tube and added 1.2 ml of pre-warmed (at 65 ^{0}C) CTAB extraction buffer 2% (1M Tris HCL , 5M NACL, 0.5M EDTA, pH 8.0) and incubated at 65°C for 1 h. An equal amount of chloroform: isoamyl alcohol (24:1) was added, mixed well by gentle inversion and centrifuged. The supernatant was transferred to a fresh tube and DNA was precipitated by adding 2/3 volume of ice cold isopropanol. After centrifugation, the pellet was washed in 70% ethanol. The pellet was dissolved in 300 μL of T10E1 buffer by keeping overnight at 4ÚC without agitation and after that kept at -20ÚC. RNA was removed by RNase (50μg/mL) treatment. The DNA concentration was checked by running samples in 0.8% agarose gel at 60 volt for 2 hours in TAE buffer along with l DNA/ECO RI + Hind III DNA ladder as standard in corner well.

Optimization of PCR conditions for RAPD analysis

For assessment of genetic diversity a total 32 RAPD decamer primers were procured from Bangalore Genei Pvt Ltd, India for initial screening. Out of 32 primers only 20 RAPD primers were amplified with desirable polymorphism as shown in Table 2. Protocol for PCR was optimized by varying the concentration of template DNA, Taq DNA polymerase and $MgCl_2$ concentration to give desirable and scorable amplification in PCR shown in Table 3.

The polymerase chain reaction was performed using thermocycler M. J. research inc PTC-200 with the following temperature profile mentioned in Table 4. PCR amplified products were loaded into individual channel of 1.2% agarose horizontal gel in TAE (0.04 M Tris-Acetate, 0.5 M EDTA, pH 8) buffer. Electrophoresis was carried out at 65 volt for 3 hours. Gels were stained with ethidium bromide (6x loading dye) of 5 ml/100ml and were observed in a transiluminator under UV light. The l DNA double digested with *Hind* III DNA and *EcoR* I was used as DNA marker for comparing the molecular weights.

Scoring of band and data analysis

Data were entered using a matrix in which all the observed bands or characters were listed. The RAPD pattern to each isolate was evaluated assigning character state to all the bands that could be observed in gel with certain primers. The character state "1" and "0" was given to the presence and absence of bands, respectively. The estimates of genetic similarity were calculated following Jaccards. The similarity

Table 1: List of egg plant genotypes used for study

S.No.	Genotype Name	S.No.	Genotype Name	S.No.	Genotype Name	S.No.	Genotype Name
1	Swarn Shobha	7	Nurki	13	Punjab Barsati	19	Brinjal Sadabahar
2	Arka Shree	8	Swarna Avilamb	14	Aruna	20	Arka Shirish
3	DBL-329	9	Swarn Pratibha	15	CHBR-2	21	Azad brinjal
4	KS-224	10	Green long cluster	16	KS-331	22	Rajendra Baigan
5	Swarn Shree	11	Ramnager Gaint	17	Arkakranti	23	Surya
6	Uttara	12	Pant Rituraj	18	BRSPS-14	24	CO-2

Table 2: List of 20 random decamer primers used for analysis genetic diversity

S.No.	Primer Name	Sequence 5'-3'	S.No.	Primer Name	Sequence 5'-3'
1	OPAG-15	CCCACACGCA	11	OPL-14	GTGACAGGCT
2	OPC-06	GAACGGACTC	12	OPL-16	AGGTTGCAGG
3	OPC-08	TGGACCGGTG	13	OPL-20	TGGTGGACCA
4	OPC-14	TGCGTGCTTG	14	OPM-12	GGGACGTTGG
5	OPC-16	CACACTCCAG	15	OPM-14	AGGGTCGTTC
6	OPF-16	GGAGTACTGG	16	OPN-08	ACCTCAGCTC
7	OPH-10	CCTACGTCAG	17	OPN-12	CACAGACACC
8	OPK-20	GTGTCGCGAG	18	OPN-14	TCGTGCGGGT
9	OPL-07	AGGCGGGAAC	19	OPV-17	CCACGGGAAG
10	OPL-08	AGCAGGTGGA	20	OPZ-20	TGTGCCCGAA

Table 3: PCR components and their concentration used for genetic diversity assessment

S. No.	Component	Concentration
1	DNA templete (25 ng/mL)	1μL
2	$MgCl_2$- 1.5mM	1.5μL
3	dNTP 0.1 mM	1.5 μL
4	Taq DNA polymerase - 1.5 unit,	0.33 μL
5	Primer	2 μL
6	Double distal water	13.7 μL
7	Total	20 μL

Table 4: PCR conditions for amplification of egg plant genotypes.

S.No.	PCR Condition	Time
1	Initial denaturation	94^0 for 4 min
2	Denaturation	94^0 for 1 min
3	Annealing	32.5^0 for 1 min
4	Extension	72^0 for 10 min
5	Hold	4^0 until do not remove

coefficients were subjected to Unweighted pair Group of Arithmatic Average (UPGMA) method of cluster analysis to group the isolates based on their overall similarities. NTSYS-PC version-2.11 software was used for cluster analysis and then the dendrogram was prepared.

RESULTS AND DISCUSSION

At present many molecular techniques are used for analyzed genetic diversity in eggplant (Prohens et al., 2005; Munoz-Falcon et al., 2008). Such studies are, however, imperative for the information that they supply for germplasm management and breeding efforts using collected genetic material. The RAPD technique provides sufficient polymorphisms for characterization of different genotypes of egg plants. The 24 genotypes were subjected to RAPD screening to detect polymorphism with 20 random oligonucleotide primers. PCR amplification with each of the primer was done twice before scoring for presence or absence of bands. The amplicon size ranging between 500 bp to 1500 bp was produced. The primers generated numbers of bands range from 6 to 1 of which only 5 primers showed polymorphism. The primer which amplified bands specific to the genotype reveals a proper genetic diversity. A high degree of polymorphism, in general, was obtained with most of the primers especially OPV-17, OPV-18 and OPZ-20 indicating a wide range of diversity at DNA level also among germplasm accessions. Similar to these kinds of observation was also observed by Khan *et al.*, 2013. They use three RAPD primers to estimate genetic diversity in five brinjal cultivars. A total of 30 bands were scored corresponding to an average of 10 bands per primer with 10 bands showing polymorphism (33.33%).One out of three primers (OPB-07) gave 50% polymorphism. Similar results was also observed by chandra and choudhary, 2014, in 10 accession of Aloe spp and found 32.8 percent polymorphism.

Similarity index

Thus a dendrogram (Fig. 1) was constructed by UPGMA method using to NTSYS-PC software (version 2.11) to measure the resulting phenotypic groups and Cluster analysis. The dendrogram showed one major clusters.

The scale of the dendrogram was between 0.27 and 1.05 with a mean value of 0.66. Dendrogram based on Nei's (1972) genetic distance UPGMA indicated segregation (grouped) of the twenty four eggplant genotypes into six main distinct clusters and in one major cluster. In the Cluster I one accession Ram Nagar Giant with included other genotypes cluster split at a Jaccard's (1908) Similarity Index (JSI) 0.28. Thus in Cluster Group-I showed lowest Jaccard's inter-varietal similarity index 28% and highest Genetic Distance 77% from other remaining genotype cluster groups.

Cluster II the genotype Rajendra Baigan showed 0.38 JSI (or 38%) and genetic distance of 67% between Rajendra Baigan and other accessions of eggplant. While in Cluster III varieties KS-224 and Swarn Avilamb formed the single group and showed 0.48 JSI or Jaccard's inter-varietal similarity index 48% and Genetic Distance-57% with the other remaining genotypes of cluster groups. Similarly in Cluster IV accessions Uttara and Green long cluster formed single group and showed 0.56 JSI or 56% JSI in % and Genetic Distance 49% with other accessions. Cluster V includes genotypes Punjab Barsati, BRSPS-14 formed a single sub-sub cluster group and have .90 JSI or 90% themselves. This single group showed 0.85 JSI and Genetic Distance 15% with sub cluster genotype Brinjal Sadabahar. This sub cluster having genotypes Brinjal Sadabahar, Punjab Barsati and BRSPS-14 (Sub-sub cluster group) separated from other accessions other sub clusters with JSI value r =0.62.

Last Cluster VI includes the one branch of Cluster VI represented by Swarn Shobha variety showed split 0.66 JSI or 66% and Genetic Distance 39% with the subcluster-1 which represents majority of the varieties. Branch Group-I of the sub cluster-I of Cluster Group-VI included Arka Shree, Arka Kranti, Arka Shirish, CO2, Swarn Pratibha, Pant Rituraj, Aruna, Nurki, CHBR-2, Surya and KS-331. Among them Pant Rituraj, Aruna, Nurki and CHBR-2 genotypes formed single group and showed 0.99 JSI or 99% Jaccard's inter-varietal Similarity Index and genetic distance was JSI value 0.06 or 6% themselves. Same similarity 99% and 6% genetic Distance was found with other genotypes group of this branch namely CO2 and Swarn Pratibha.

CO-2, Swarn Pratibha, Pant Rituraj, Aruna, Nurki, CHBR-2 showed 88% similarity and Genetic Distance of 17% with other remaining genotypes of this branch namely Arka Shree, Arka Kranti and Arka Shirish. Arka Shrish and Arka kranti divided in single group at 0.99 JSI or showed 99% similarity and Arka shree lonely accession of that sub cluster that showed 86% similarity and Genetic Distance of 14% with this single group.

In other side sub-sub cluster group's Surya and KS-331 accessions also form single group and showed 0.90 JSI similarity with themselves and 0.78 JSI similarity between this single group and remaining genotypes group of this branch. In branching group-2 of sub cluster I of Cluster Group-6, DBL-329 accession showed 84% similarity or 0.84 JSI with a single group accession which is formed from accessions Swarn Shree and Azad Brinjal having JSI 0.99 or 99% similarity between them. They showed 70 % similarity with other accessions of branch group-I.

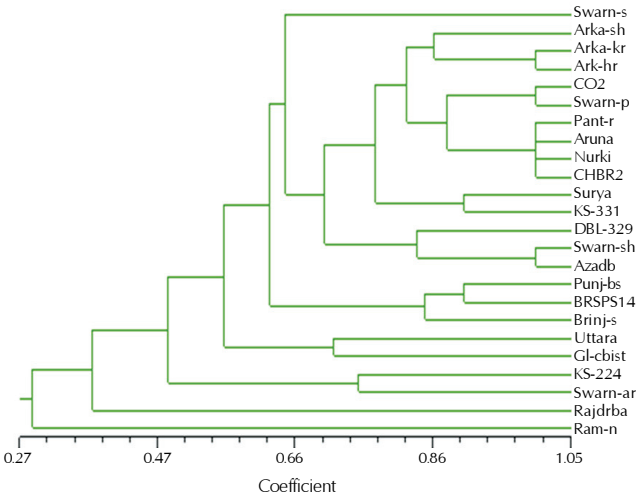

Figure 1: Dandrogram contructed with UPGMA Clustering method of genotypes of Solamun melongena RAPD markers

Thus UPGMA Dendrogram of 24 *Solanum melongena* accessions generated based on genetic distance metrix used for Cluster analysis concluded that ten genotypes namely Arka Kranti, Arka Shirish, CO-2, Swarn Pratibha, Pant Rituraj, Aruna, Nurki, CHBR-2, Swarn Shree and Azad Brinjal of major cluster group -VI showed maximum Jaccard's intervarietal similarity Index (JSI) of 0.99 or 99% and lowest Genetic Distance of 6% than other genotypes of various cluster groups while genotype Ram Nagar Giant of cluster group –I showed lowest Jaccard's intervarietal Similarity Index (JSI) of 0.28 or 28% and maximum Genetic Distance of 77% with other genotypes of other cluster groups. The level of polymorphism depicted in the present study was high which is revealed by the coefficient of variation. Similarly, Soni and Khanorkar in 2013, also classified maize genotype in two different cluster based on RAPD analysis and found significant genetic variability.

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