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Assortment of Genetic Diversity in Brinjal *(Solanum melongena)* Genotypes Using ISSR markers

KEYWORDS: Brinjal, DNA extraction, PCR, Electrophoresis.

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ABSTRACT *Brinjal is an important tropical as well as subtropical vegetable crop, mostly used for different types of recipes. Also have a medicinal property which most important for human health. This study aimed to determine the genetic diversity between Brinjal varieties. For this purpose, eleven primers were used to characterize five Brinjal varieties under cultivation in different state of India as contrasting genotype. 69 scorable bands were generated among 44 comprised polymorphic markers, with an average of 4 polymorphic bands per primer. In the generated dendrogram the accession were placed in cluster, where cluster A include only one genotype from MPKV India (Pusa Purple long) and cluster B is biggest cluster which include three genotype. This molecular marker tools are widely used in plant research such as phylogenic studies as well as in cultivar identification and germplasm management. The selected primers were used for the first time in Brinjal, representing valuable tools for future evaluations, with emphasis to diversity characterization and genetic mapping.*

1. Introduction:-

Brinjal (Solanum melongena L.) of Solanaceae family is one of the widely used vegetable crops by most of the people and is popular in many countries viz., central south and south East Asia, some part of Africa and Central America (Grubban1977). It is an important vegetable due to its nutritive value, consisting of mineral like iron, phosphorous, calcium, and vitamin like A, B, and C. Unripe fruits are used primarily as vegetable in the country. It also used as raw material in pickle making and dehydration industries (Singh et.al., 1992) and is an excellent remedy for those suffering from liver complaints. It is used in ayurvedic medicine for curing diabetes and also as a good appetizer. It is good aphrodisiac, cardiotonic, laxative, mutant, and reliever of inflammation.

In world, Brinjal occupies an area of 1.6 million ha with production of 41.84 million tones per ha (FAO 2010). According to FAO, production of Brinjal is highly concentrated, with 90% of output coming from five countries. China is the top producer (58% of world output) and India is second (25% of output), followed by Egypt, Iran, and Turkey. In the world more than million species are cultivated. In that some species are self pollinated and some hybrids. The identification of crop plant has become increasingly important of the documentation of genetic resource and to protect of the breeder interests. For the recipes and food industries, this is especially important different varieties of Brinjal have widely different qualities and use characteristics. Farmer needs positive identification for the protection of their proprietary right on varieties. Process must be assured of varietal identity and that it is free from mixture. Examination of grain morphological characteristics is the standard method of identification, but not all of them can be distinguished on this basis.

Hence researchers develop new technology that is Molecular marker technology. Molecular markers have been proved to be valuable tools in the characterization and evolution of genetic diversity within and between species and population. Genetic diversity is different forms of genotype and occurs as a result of change in genetic structure (WEB_2 2007). It potentially leads to specification in the long term due to the process of evolution (Raven et. al. 1990, WEB_2 2007). Diversity in genetic composition is the basic feature which increases chance of survival for individual and population during natural selection (WEB_2 2007). It has been showed that different markers might reveal different class of variation. It is correlated with the genome fraction surveyed by each kind of marker, their distribution throughout the genome and the extend of the DNA target which is analyzed by each specific assay. The advent of the polymerase Chain Reaction (PCR) favored the development of different molecular techniques such as Inter Simple Sequence Repeat Polymorphic DNA (ISSR), Random amplified of Polymorphic DNA (RAPD), Sequence tagged site (STS), Random amplified microsatallite DNA(RAMP)) and Single Sequence Repeat (SSR).

Out of these techniques, ISSR have several advantages such as better result, simplicity of use, low cost and the use of small amount of plant material. ISSR assay has been used by several groups as efficient tools for identification of marker linked to agronomically important traits. ISSR markers are widely used in plant research such as phylogenic studies, genetic mapping, and Population genetics studies as well as in cultivar identification and germ plasm management. The application of ISSR and their relaned modified markers in variability analysis and individual specific genotype has been largely carried out. ISSR marker had been used in potato, barley, wheat been and sugarcane. In this study, we evaluated the assortment of genetic diversity and relationship between brinjal specimen which are cultivated in different state of India using ISSR marker, in order to establish a base line to assist future conservation and breeding programmes of this species. Also we aim to report the usefulness of ISSR markers for the assortment of genetic diversity and relationship among brinjal specimen.

2. Material and method

2.1. Plant Material.

Five brinjal (Solanum melongena L.) specimen selected from IARI, New Delhi and MPKV, Maharashtra (India) were used in this study. The specimen origin and yield are listed in **Table 1.**

2.2. DNA Extraction.

DNA was isolated from young leaves tissue using CTAB (centyl-trimethyl-ammoniumbromide) protocol. DNA concentration was determined both by 1% agarose gel and spectrophotometric method at 260nm.

2.3. ISSR-PCR analysis.

The ISSR amplification reaction contained 10ng of genomic

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DNA, 2.5µl 10 x buffer, 2.5mM $Mgcl_2$ 40mM each dNTPs (Fermentas), 10µM primer and 3U Taq DNA polymerase (Invitrogen), with the final volume adjusted to 20µL with H_2O. The amplification reaction was carried out in an Eppendorf Mastercycler. The amplification programme comprising of initial denaturation step at 94^{0}C for 5 min, followed by 35 cycles, each consisting of a denaturation step of 30s at 94^{0}C, annealing at 45^{0}C for 1 min, and an extension of 1 min at 72^{0}C.

PCR was terminated with final extension of 5 min at 72^{0}C. ISSR reaction product were separated on 10% horizontal agarose gel, in 1 X TAE buffer with 5 voltage V/cm and visualized under ultraviolet light after staining in 0.5µg/ml ethidium bromide in gel documentation system and captured image of each gel. The 100bp DNA ladder plus molecular weight marker was used to compare the molecular weight of amplified product.11 ISSR primers previously selected for barley, rice, wheat and potato, these 11 ISSR primer used for assortment of genetic diversity of Brinjal specimen **(Table 2.)**

2.4. Data analysis.
Polymorphic ISSR markers were scored as binary data presence (1) or absence (0).The weak and spurious bands were excluded from the analysis. The genetic similarity among the varieties was calculated by Jaccard similarity coefficient using NTSYS-pc analysis software (ROHLF 1998).A dendrogram was constructed based on genetic distance using neighbor joining method. Principal component analysis was conducted on the basis of correlation matrix for banding pattern generated from ISSR profile and computed using a NTSYS-pc analytical package.

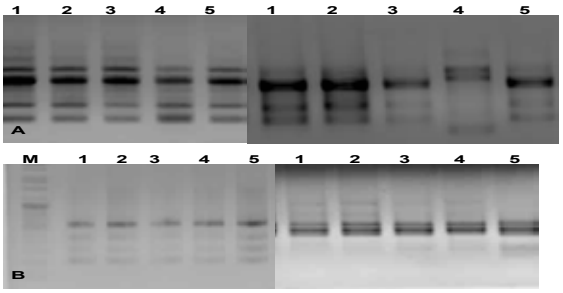
3. Result and Discussion.
3.1 PCR analysis.
The ISSR analysis, carried out in 5 varieties produced 69 bands, from which 44 were polymorphic among the Brinjal varieties. All primers amplified fragments, with a number of amplicons varying from 7 (primer 820) to 1(primer815) fragment per reaction, with size varying from ~ 200bp to 1.2kb (Table.3). No single band was specific to any individual varieties.

3.2. Genetic distance analysis.
The numerical data were generated from ISSR profiling of 5 genotype of Brinjal variety and used to compute the genetic distance based on Jaccard Index **(Table.4)**. The maximum distance was observed between genotype 1 and 5 (0.468). The second maximum distance was observed between genotype 1 and 2 (0.394). As compared to other genotype minimum distance was observed between 2 and 3 (0.112).

3.3. Cluster analysis.
The neighbor joining method generated a dendrogram with two main clusters, (Cluster 1 and cluster 2).Cluster 1 include only one variety from IARI. New Delhi and 2 include four varieties from MPKV. Maharashtra. Genotype 1 placed in separate group from other four. Genotype 2(Pragati) and 3(Vaishali) were place together.

3.4. Correlation Coefficient Analysis.
The correlation coefficient of 5 genotype of brinjal was calculated based on ISSR profile. From this analysis, the highest and lowest correlated brinjal species were observed. The genotype 2 (Pragati) and genotype 3 (Vaishali) was highly correlated. Second highest correlated genotype was genotype 3 (Vaishali) and genotype 5 (Pusa Purple long). Similarly as compared to other lowest correlated genotype was genotype 1(Pusa Purple long) and genotype 3(Vaishali).

4. Acknowledgements.
To IARI, New Delhi and Mahatma Phule Krushi Vidyapeeth, Rahuri, Maharashtra for providing varieties.



Figer.1. Examples of ISSR marker amplification result in brinjal. (A) Amplicons using primer ISSR 820 & ISSR 812 (B) Amplicons using primer ISSR 842 & ISSR 816

Figer.2.Cluster 1-Pusa Purple Long(1), Cluster 2- Pragati(2), Vaishali(3), Krishna(4), Mangirigota(5)

Table1.
(MPKV- Mahatma Phule Krushi Vidhyapeeth Rahuri, IARI- Indian Agriculture Research Institute New Delhi)

Sr. No	Species	Parentage	Institute/ University	Yield
1	Pusa Purple Long	Batia Cultivar Of Punjab	IARI, New Delhi	310
2	Pragati	Vaishali X Arka Kusumakar	MPKV,Maharashtra	350
3	Vaishali	Arka Kusumakar X Manjari Gota	MPKV,Maharashtra	350
4	Krishna	Fuji Hybrid	MPKV,Maharashtra	480
5	Manjari Gota	Local Germplasm	MPKV,Maharashtra	250

Table2.
ISSR primers were obtained from the University of British Colombia (Ayres and Strong 2001, Fernández et al. 2002, Jeung et al. 2005) R = (A,G); Y = (C,T); D = (A,G,T) (i.e. not C); H = (A,C,T) (i.e. not G), V = (A,C,G) (i.e. not T). Legend for abbreviations: Temp. = Annealing temperature; # = number; Polym. = Polymorphic/Polymorphism.

S.No	Primer	Sequence 5'- 3'
1	UBC 807	AGAGAGAGAGAGAGAGT
2	UBC 815	CTCTCTCTCTCTCTCTTG
3	UBC 820	GTGTGTGTGTGTGTGTC
4	UBC 812	GAGAGAGAGAGAGAGA
5	UBC 842	GAGAGAGAGAGAGAGAYG
6	UBC 816	GAGAGAGAGAGAAYT
7	UBC 840	GAGAGAGAGAGAAYT
8	UBC 810	GAGAGAGAGAGAGAGAT
9	UBC 848	CACACACACACACACARG
10	UBC 818	CACACACACACACACAG
11	UBC 841	GAGAGAGAGAGAGAGACC

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Table 3.
The ISSR analysis, carried out in 5 varieties produced 69 bands, from which 44 were polymorphic among the Brinjal varieties.

S.No	Primer	Total number of bands	polymorphism %
1	UBC 807	6	100
2	UBC 815	1	100
3	UBC 820	8	87.5
4	UBC 812	7	85.7
5	UBC 842	7	71.4
6	UBC 816	7	57.1
7	UBC 840	9	55.5
8	UBC 810	6	50
9	UBC 848	5	40
10	UBC 818	5	40
11	UBC 841	8	37.5

Table 4.
Genetic distance analysis.
The numerical data were generated from ISSR profiling of 5 genotype of Brinjal variety and used to compute the genetic distance based on Jaccard Index.

Genotypes ®/¯	1	2	3	4	5
1	0				
2	0.394	0			
3	0.457	0.112	0		
4	0.369	0.328	0.36	0	
5	0.468	0.197	0.172	0.308	0

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