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Screening and Identification of Molecular Marker for Fingerprinting of Brinjal Hybrids & its Parental Lines

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ABSTRACT

Brinjal (Solanummelongena) belonging to the family solanace brinjal fruit are a good source of calcium, phosphorus, iron and vitamins particularly B group. In addition Brinjal have much medicinal value .In can block free radicals, helps to control cholesterol level. The geniviness of a hybrid purity is one of the most important characteristics of good quality seeds. The confirmation of genetic purity in seeds is very important for identification of genetic and as well as at a commercial level. The recent advances in molecular marker proves its efficiency in the genetic purity analysis than conventional method (GOT).The main objective of this study to identify the genetic purity of the brinjal hybrids and its parental line with the help of molecular markers. In the present research 18 ISSR and 10RAPD primers were selected for the study of 3 EGG PLANT hybrids (AS 1001, AS1002, AS1003)and its parental line. All primers shows good amplification but only one ISSR & one RAPD primers produced unique fingerprint across the hybrids. ISSR primer UBC 807 for (ASH1002,ASH1002)likewise RAPD primers B18 for ASH1003 thus our study showed that aid of molecular marker are more reliable, highly efficient and reproductive for assessing fingerprinting of brinjal commercial hybrid seed`s with more accuracy.

Keywords : Brinjal, ISSR, RAPD, Polymorphism, Fingerprinting

I. INTRODUCTION

Brinjal (*Solanummelongena* L.), also known as Aubergine, Brinjal , Eggplant, Guinea squash or poor man's crop is one of the common, popular and principal vegetable crops grown in different parts of the world . It is one of the non-tuberous species of the night shade family Solananceae (Kantharajah and Golegaonkar, 2004) with basic chromosomal number 2n = 24. India is probably the centre of its origin and is native to Indian sub-continents (Gleddie *et al.,* 1986b). The four major brinjal producing countries are China, India, Egypt and Turkey. Of the total world production India is the second largest producer, which is estimated of 43.17 million tons (NHB, 2011). In India, it occupies the third position among vegetable crops and it is cultivated throughout the country including tropical, subtropical and temperate

regions. The objective of the present study to identify molecular markers for genetic purity analysis of commercial Brinjal hybrids ABH1001, ABH1002, ABH1003 and their parental lines using agarose gel electrophoresis and to develop a more efficient method for genetic purity analysis of Brinjal hybrids than conventional GOT method.Molecular markers have many advantages such as polymorphism can be obtained in abundance, selected at any growth stage, no pleiotrophic effect, environmental effect is very low 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 amongelite breeding lines, populations and also in wild species with high precision. Molecular markers reflect the difference in DNA level without environmental impacts, and thus have great advantages in seed purity identification (Nicholas *et al.,* 2012). Inter-simple sequence repeat (ISSR) and Random amplified polymorphic DNA (RAPD) (Nunome*et al.*, 2001) were extensively used for the genetic purity testing of brinjal.

II. Materials and methods

Plant material

The study referred to the DNA fingerprinting of Brinjal hybrid using molecular markers was conducted at Aditya Biotech Lab and Research, Raipur, Chhattisgarh. The plant material collected for the study was collected from Aditya seed Pvt. Ltd, Raipur, Chhattisgarh. Young and fresh leaves of Brinjal hybrids and their parental lines (Table No.1) were collected from the field for the extraction of DNA.

Extraction of genomic DNA

Extraction of genomic DNA was carried out using modified CTAB (N-cetyltrimethyl ammonium bromide) method ((Zhu *et al.,*2010). The extracted DNA was diluted in MQ and stored at -20°C for further use. The DNA was quantified using 0.8% agarose gel in 1X TAE buffer and photographed under UV light of Bio-Rad gel documentation system.

ISSR and RAPD PCR amplification and electrophoresis

Eighteen ISSR primers and eight RAPD primers were selected for the PCR amplification of DNA samples. 8 from 18 ISSR primer and 3 from 8 RAPD primers scored polymorphism (Table No.2) among the parental lines. Primer showing high polymorphism was selected for the fingerprinting of respective hybrids as their level of polymorphism was clear and distinguishable.

PCR reaction were conducted in final volume of 20µl containing 1µl(40ng) of DNA sample, 2µl of 10X PCR buffer, 0.5µl $MgCl_2$ (25mM), 1 µl dNTP's (5mM), 1µl BSA(10mg), 0.3µl Tween 20, Primer (ISSR/RAPD) 2.0µl/ 1.5µl (3-5 pM), 0.2µl(1Unit) of Taq polymerase. The PCR protocol consists of initial denaturation at 94ºC for 5min, followed by 38 cycles at 94ºC for 20sec, annealing for 30sec at the Tm of primer, 72ºC for 1min 30sec and final extension step at 72ºC for 5min was conducted in Thermo cycler (Applied Biosystem). The PCR product was analyzed in 2% agarose gel in 1X TBE buffer along with 100-1000bp molecular weight marker and photographed under UV light of Bio-Rad gel documentation system.

Result

Four Brinjal hybrids and their parental lines were analyzed for ISSR and RAPD primers polymorphism. All the ISSR and RAPD primer had good amplification. Out of eighteen ISSR primer, eighteen ISSR Primer and ten RAPD primers were scorable on agarose gel and showed polymorphism in parents. On the basis of the parental polymorphism one primer from ISSR i.eUBC 807 and two from RAPD i.eB20 and B18 were found informative for the fingerprinting of hybrids. These selected markers amplified unique fingerprinting for the hybrid which can therefore made iteffective to distinguish them from one another. On the basis of morphological observation and the banding pattern observed using

ISSR and RAPD marker confirmed the parent line with their respected hybrids.

The ISSR marker UBC 807 demonstrated amplified alleles are of size 900bp on male line AS Shaymli and 1200bp on female line (AS RJ) for Hybrids ABH1001 and vice versa for Hybrid ABH1002, exhibited both the alleles of parental lines. So,the heterozygosity of the hybrid with the presence of both the alleles of parent with similar band size *i.e.*900 bp and 1200 bp was confirmed (Fig.1).

Hybrid ABH 1003 was identified and distinguished with the RAPD primers B18, as below shown in (Fig.2). The marker having an amplicon of size 850 bp& 800 bp on female line i.e AS4 and 950bp amplicon on male line i.e AS RJ.The hybrid showed both the amplicons different size of 800bp, 850bp and 950 bp. Thus, this confirms the genuine nature of hybrids.

Discussion

As our study showed that the aid of molecular marker are more reliable,very efficient and reproductive for assessing fingerprinting of brinjalcommercial hybrid seed`s with more accuracy.Because for seed production assessment of seed purity is most important. Conventionally,as morphological methods used for the access of purity test.These methods were very tedious, and also requires large area and it is also very time consuming and often the result is not unequivocal identification of genotype. Recent development of molecular marker has suggested for genetic purity test as they are access the DNA level identification. So the molecular marker based techniques has now become the cornerstone to the improvement of global agricultural production. ISSR and RAPD markers were reported useful for the assessment of purity test of brinjal. And Agarose gel electrophoresis was used for the study of amplification as this method is comparatively easier and also affordable. The agarose gel electrophoresis also showed that the ISSR and RAPD markers were valuable for the genetic purity test of Brinjal. And on the basis of stable, distinct and polymorphic amplification observed on agarose gel RAPD primer B18,ISSR primer UBC 807 were scored for purity assessment of brinjal.

Figures and Tables

Table No.1 Name of brinjal hybrids and parental lines used.

S. No.	Parent line		Hybrid	Source
	Female	Male		
1	AS RJ	AS Shaymli	ASH 1001	Fields of Aditya Seeds Pvt Ltd, Raipur (C.G.)
2	AS Shaymli	AS RJ	ASH 1002	
3	AS 4	AS RJ	ASH 1003	

Table No.2 ISSR and RAPD primers, its sequence which showed polymorphism in parental line

Primer Name		Sequence	Parental line (Polymorphism observed)
ISSR	UBC 807	AGAGAGAGAGAGAGAGT	AS RJ
	UBC 812	GAGAGAGAGAGAGAGAA	AS Shaymli
	UBC 815	CTCTCTCTCTCTCTCTG	AS RJ
	UBC 807	GTGTGTGTGTGTGTGTC	AS RJ, AS Shaymli
	UBC 822	TCTCTCTCTCTCTCTCA	AS 4
	UBC 827	ACACACACACACACACG	AS Shaymli
	UBC 829	TGTGTGTGTGTGTGTGC	As 4
	UBC 847	CACACACACACACACARC	AsShaymli
RAPD	B18	GAGAGCCAAC	AS 4, AS RJ
	B20	GGACCCTTAC	AS RJ, AS Shaymli

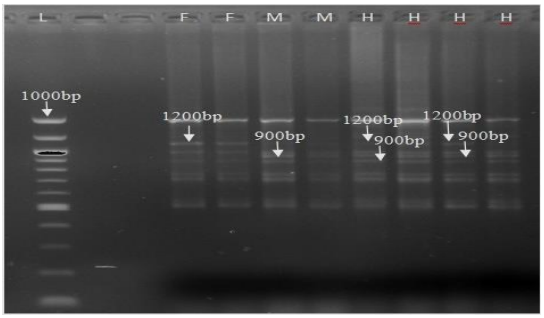

Figure No.1 Amplification result of ISSR primer UBC 807 against parental line (AS RJ and AS Shaymli) and its hybrid (ASH 1001 and ASH 1002). Lane L represents 100-1000bp ladder, Lane F represents female line AS rj and AS shaymli (amplification size 1200bp), lane M indicates male line AS RJ and AS Shaymli (amplification size 1200bp) and lane H represents hybrid ABH 1001 and ABH 1002 (amplification size 1200bp, 900bp)

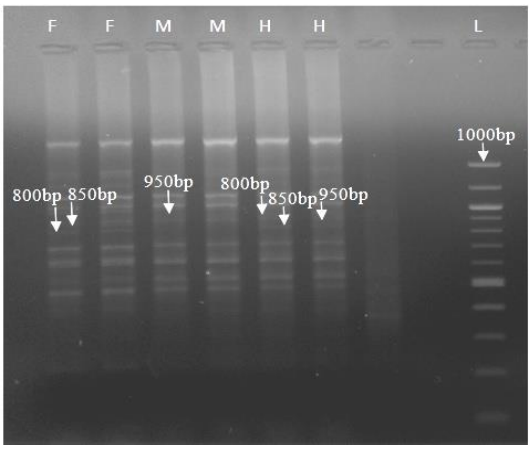

Figure No.2 Amplification result of RAPD primer B18 against parental line (AS 4and AS RJ) and its hybrid (ABH 1003). Lane F represents female line AS 4 (amplification size 800bp and 850bp),lane M indicates male line AS RJ (amplification size 950bp), lane H represents hybrid ABH 1003 (amplification size 800bp, 850bp and 950bp) and Lane L represents 100bp ladder(100bp-1.5kb)

III. Conclusion

So our study can be concluded that ISSR and RAPD markers can be efficiently used for the screening and identification of brinjal hybrid and also its parental lines. The use of molecular marker for the purity test of crop can be a born for all seed industry as the rate of accuracy is very high. It can also be said that these methods of seed purity test can also be more useful as it eradicates the conventional method of seed purity test which is more laborious and time consuming.

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