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

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Abbreviation: **CTAB**-N-cetyl trimethyl ammonium Bromide; **GOT**-Grow out test; **ISSR**-Inter-simple sequence repeat; **RAPD**-Random amplified polymorphic DNA

A B S T R A C T

Brinjal is a vegetable that is extensively grown in India. The maintenance of genetic purity of brinjal plant is a matter of great concern for the breeders. The recent advances in molecular markers prove its efficiency in the genetic purity analysis. Fingerprinting with molecular markers allows precise and rapid variety identification. The genuiness of a hybrid is one of the most important characteristics of good quality seed. Conventionally used grow out test (GOT) for genetic purity analysis requires use of morphological differences between true hybrids and off types which is not always apparent and can't be recognized easily. Further, morphological traits are costly, tedious to score and very sensitive towards environment. Eight parental lines of brinjal (*i.e.* AB54, AB73, AB52, AB 04) were used for production of 4 hybrids. Total 18 ISSR and 8 RAPD primers were selected for the study of 8 parental lines, among them 8 ISSR and 3 RAPD primers were found to be polymorphic across the parental lines, out of which 1 ISSR and 2 RAPD primers produced unique fingerprinting across the hybrids. The ISSR marker, UBC 820 amplified alleles specific to different parental lines for hybrids (ABH1001 and ABH1002), likewise RAPD primer B18 for ABH 1003 and B5 for ABH 1004. Thus, our study showed that aid of molecular markers are more reliable, highly efficient and reproducible for assessing fingerprinting of brinjal commercial hybrid seeds with more accuracy.

Introduction

Brinjal (*Solanum melongena* 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 (Bletsos *et al.*, 2003). 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. Molecular markers have many advantages such as polymorphism can be obtained in abundance, samples can be 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 among elite 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. Inter-simple sequence repeat (ISSR) markers are PCR based amplification method that is used to differentiate between closely related individuals. This class of molecular markers is based on inter-tandem repeats of short DNA sequences. These regions lie within the microsateliite repeats and offer great potential to determine intra-genomic and inter genomic diversity compared to other arbitrary primers, since they reveal variation within unique regions of the genome at several loci simultaneously. They exhibit specificity of sequence-tagged-site markers (Zietkiewicz *et al.*, 1994 and Goodwin *et al.*, 1997) and it does not require prior knowledge of DNA sequence for primer design (Zietkiewicz, 1994). The primer used in ISSR analysis gives a wide range of possible amplification products based on any of the SSR motifs (di, tri-, tetra- or penta- nucleotides) found in microsatellite loci. These primers can be anchored to genomic sequence making either side of the targeted simple sequence repeats (Zietkiewicz *et al.*., 1994). ISSR markers have been proved to be useful as novel DNA markers in studies on purposing plant improvement, such

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as genomic fingerprinting, phylogenetic analysis, gene tagging, etc.

Genetic variabilities in brinjal have successfully examines by RAPD methods in different countries (Karihaloo and Gottlieb, 1995; Singh *et al*., 2006; Yasmin *et al.,* 2006; Demir *et al.,* 2010; Ali *et al*., 2011; Sharmin *et al.,* 2011; Laila *et al*., 2012; Khalil *et al.,* 2013). RAPD technique is widely used as it is easy and inexpensive. The technique uses amplification of DNA fragments by polymerase chain reaction (PCR) using primers with arbitrary sequences for rapid screening of polymorphisms (Sobral and Honeycutt, 1993). They are particularly suitable for less well-known species because they can be applied without prior knowledge of DNA sequence information. These markers are considered to be dominant markers and can be efficient in the routine assessment of variety identification, hybrid seed purity, and genetic diversity and in analyzing genetic relationships (Nunome *et al.,* 2001).

The objective of the present study to identify molecular markers for genetic purity analysis of commercial Brinjal hybrids ABH1001, ABH1002, ABH1003, ABH1004 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.

Materials and Methods

Plant material

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

Extraction of genomic DNA

Extraction of genomic DNA was carried out using modified CTAB (N-cetyl trimethyl 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.

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 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.

Results

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 18 ISSR primer, 11 ISSR Primer *i.e.* UBC 807, UBC 811, UBC 812, UBC 813, UBC 815, UBC 820, UBC 822, UBC 824, UBC 827, UBC 829, UBC 847, UBC 883 and 5 RAPD primers *i.e* B5, B11, B18, B3, B20 primers were scorable on agarose gel and showed polymorphism in parents. On the basis of parental polymorphism one primer from ISSR (UBC 820) and two from RAPD (B5 and B18) were found informative for the fingerprinting of hybrids. These selected markers amplified unique fingerprinting for the hybrid which can therefore made it effective to distinguish them from one another. On the basis of morphological observation and polymorphisms between parents aware used as a marker for hybrids identification.

The ISSR marker UBC 820 demonstrated amplified alleles of size 1200bp on female line (AB73) and 900bp on male line (AB52) and Hybrids (ABH1001 and ABH1002) exhibited both the alleles of parental lines. Thus, the heterozygosity of the hybrid with the presence of both the alleles of parent with similar band size *i.e.*1200bp and 900bp was confirmed (Fig.1).

Hybrid ABH 1003 was identified and distinguished by the RAPD primers B18, as shown in (Fig.2). The marker had an amplicon of 850bp & 800 bp on female line (AB73) and 950bp amplicon on male line (AB54).The hybrid showed both the amplicons at 800bp, 850bp and 950 bp. Thus, this confirms the genuine nature of hybrids.

Similarly, hybrid ABH 1004 was identified and distinguished by the RAPD primers B5, as shown in (Fig.4). The marker had an amplicon of 800bp on female line (AB54) and 700bp amplicon on male line (AB73).The hybrid showed both the amplicons at 800bp and 700 bp. Thus, the presence of both female and male parent alleles indicated the result of crossing between two parents (Hybrid).

Discussion

For seed production assessment of seed purity is most important. Conventionally, morphological methods used for the access of purity test. These methods were very tedious, requires large area and it is very time consuming and often the result is not unequivocal identification of genotype. Hence, a recent development in molecular markers has been suggested for genetic purity testing, since they are used to precisely assess the genotype, and not the phenotype (R.M. Sundaram *et al*., 2007).The molecular marker based techniques has 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.

Agarose gel electrophoresis was used for the study of amplification as this method is easier and affordable. The result obtained was valuable for the assessment of distinct and polymorphic ISSR and RAPD marker amplification. The agarose gel electrophoresis showed that the ISSR and RAPD markers were valuable for the genetic purity test of Brinjal. On the basis of stable, distinct and polymorphic amplification observed on agarose gel ISSR primer UBC 820 and RAPD primer B5&B18 were scored for the purity assessment of brinjal.

Table – 1: Name of brinjal hybrids and parental lines used

S. No.	Parent line		Hybrid	Source
	Female	Male		
1	AB73	AB52	ABH 1001	Fields of Aditya Seeds Pvt Ltd, Raipur (C.G.)
2	AB52	AB73	ABH 1002	
3	AB73	AB04	ABH 1003	
4	AB54	AB73	ABH 1004	

Table – 2: ISSR and RAPD primers, its sequence which showed polymorphism in parental line

Primer Name		Sequence	Parental line (Polymorphism observed)
ISSR	UBC 807	AGAGAGAGAGAGAGAGT	AB73
	UBC 812	GAGAGAGAGAGAGAGAA	AB73
	UBC 815	CTCTCTCTCTCTCTCTG	AB52
	UBC 820	GTGTGTGTGTGTGTGTC	AB73, AB52
	UBC 822	TCTCTCTCTCTCTCTCA	AB73
	UBC 827	ACACACACACACACACG	AB52
	UBC 829	TGTGTGTGTGTGTGTGC	AB73
	UBC 847	CACACACACACACACARC	AB52
RAPD	B5	TGAGCGGACA	AB73, AB54
	B18	GAGAGCCAAC	AB73, AB04
	B20	GGACCCTTAC	AB73, AB52

Figure – 1: Amplification result of ISSR primer UBC 820 against parental line (AB73 and AB52) and its hybrid (ABH 1001 and ABH 1002). Lane L represents 100-1000bp ladder, Lane F represents female line AB73 and AB52 (amplification size 1200bp), lane M indicates male line AB52 and AB73 (amplification size 1200bp) and lane H represents hybrid ABH 1001 and ABH 1002 (amplification size 1200bp, 900bp)

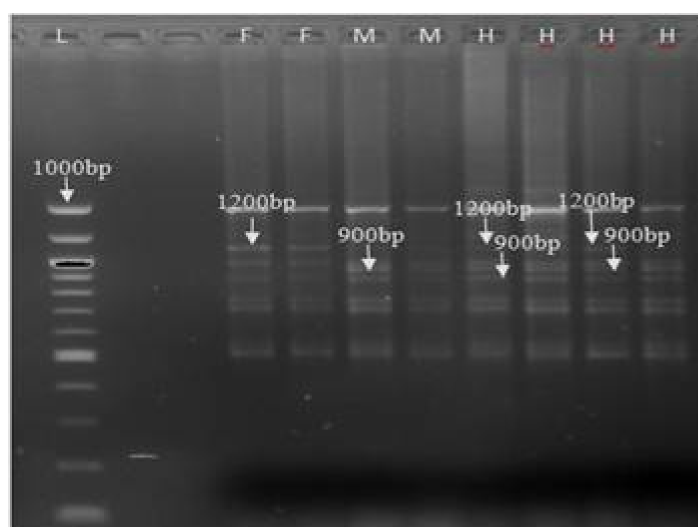


Figure – 2: Amplification result of RAPD primer B18 against parental line (AB73 and AB04) and its hybrid (ABH 1003). Lane F represents female line AB73 (amplification size 800bp and 850bp), lane M indicates male line AB04 (amplification size 950bp), lane H represents hybrid ABH 1003 (amplification size 800bp, 850bp and 950bp) and Lane L represents 100bp ladder(100bp-1.5kb)

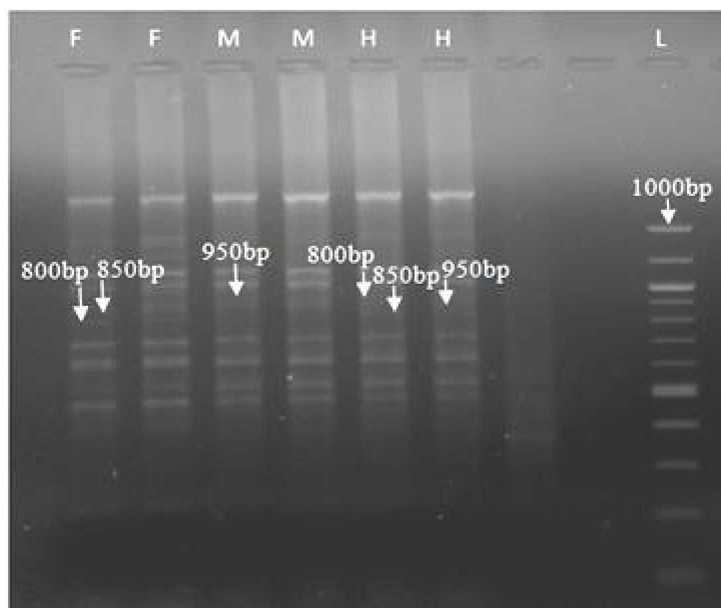
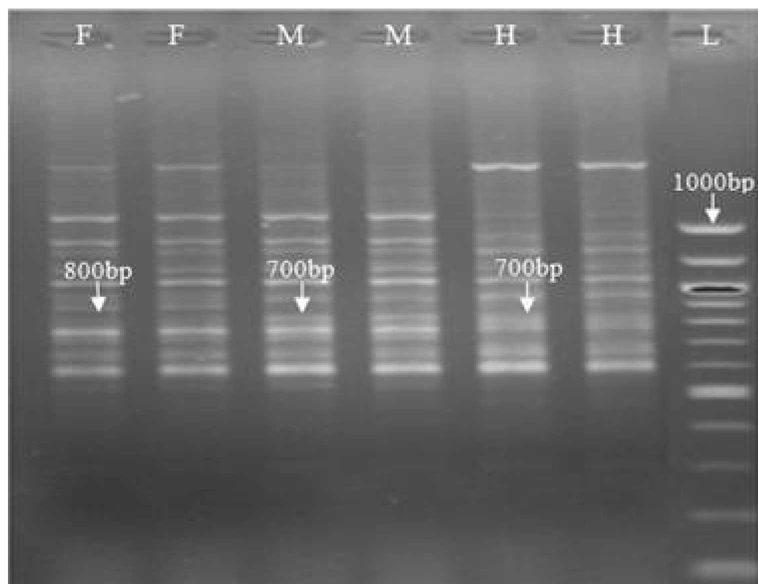


Figure – 3: Amplification result of RAPD primer B5 against parental line (AB54 and AB73) and its hybrid (ABH 1004). Lane F represents female line AB52 (amplification size 800bp), lane M indicates male line AB73 (amplification size 700bp), lane H represents hybrid ABH 1004 (amplification size 800bp, 700bp) and Lane L represents 100bp ladder (100bp-1.5kb)



Conclusion

From this study it can be concluded that ISSR and RAPD markers can be efficiently used for the screening and identification of brinjal hybrid and its parental lines. DNA markers are more accurate in DNA fingerprinting as the marker analyses the DNA leveled polymorphism. The use molecular marker for the purity test of crop can be a boon for seed industry as the rate of accuracy is very high. It can also be stated that these methods of seed purity test can be more useful as it eradicates the conventional method of seed purity test which is more laborious and time consuming.

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