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Molecular assessment of hybridity in F_1 (*Luffa acutangula* × *Luffa cylindrica*) through SSR marker

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Abstract

An experiment was carried out to test the hybridity of offspring of *Luffa acutangula* and *Luffa cylindrica* through SSR marker. Experiment was conducted at the laboratory of Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. Hybridity test through molecular maker is a new concept in the field of applied molecular biology in agriculture. The present finding indicated that SSR primer can be used for detection of polymorphism among the parents of any hybrid. The identified polymorphic primer can be used for hybrid identification for commercial purpose. All the seed companies producing and selling hybrid seeds can use this molecular technique. It will save money, time and manpower than traditional methods of hybridity test. Seven ridge gourd and three sponge gourd genotypes were used as parents. For hybridity test, initially different SSR primers were used to establish polymorphism among the parents of each hybrid. The identified polymorphic primers were used to screen the hybridity test. It was noticed that three primers *viz.* BoG-21, BoG-76 and BoG-117 showed polymorphism in the parents and their corresponding hybrid. It was noted that, a 550bp DNA fragment was amplified in male parent and hybrid of Bogra×RIG 47-2 which was absent in female genotype. 200 bp DNA fragment was amplified in male parent and hybrid in case of Bogra×BARI Jhinga-1. This DNA band can be used for large scale screening of that specific hybrid variety for seed purity in commercial purpose. The parents of one hybrid (Faridpur×KRG 02) showed monomorphic DNA band and one of the SSR primers viz. BoG 118 was not amplified in any ridge gourd and sponge gourd genotype under investigation. Hybridity (*Luffa acutangula* × *Luffa cylindrica*) was identified by the same DNA fragment that found in male parents and F_1 offspring.

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Introduction

Ridge gourd (*Luffa acutangula* L., n=26) and Sponge gourd (*Luffa cylindrica*) are two most important cucurbitaceous vegetables in Bangladesh available during summer season. Interspecific hybridization allows a transfer of genes of interest between different species (Bosland and Votava, 2000). Characteristics of the offspring of the compatible parent can be confirmed by two methods. One is conventional method another is molecular technique. The conventional method for estimating hybrid seed purity or hybridity is known as Grow-Out Test (GOT).The hybridity test done in natural field condition. The seed of parent1 (male), parent2 (female) and hybrids are sown in ideal condition. The morphological data are recorded in all the hybrid samples and then it is compared with parental line. There are some disadvantages involved in grow-out test such as- some morphological characteristics need to be estimated in adult stage and are influenced by environmental factors, take more time to complete. Seeds have to be stored safely before they are released to farmers. The investment used in producing the seeds is high. Huge amount of land, labor and cultivation cost are involved. Seed viability is reduced over the period of time.

Hybridity test through molecular technique for assessing purity of parental lines is done by DNA markers. It has several advantages over conventional method, such as not influence by environment, high power of discrimination among closely related individual, polymorphism establishment within DNA level, more accurate, reliable and exact blue print of genetic makeup can be achieved. We can use molecular technique at the early stage of crop such as leaf stage and we need not to wait for maturity. Molecular markers provide an efficient means to link phenotypic and genotypic variations (Varshney *et al.*, 2005). These methods are being very rapidly adopted by the researchers of all over the world for the crop improvement. Molecular markers present an efficient tool for fingerprinting of cultivars, and assessment of genetic similarities and relationships (Vilanova *et al.*, 2012). DNA markers are accepted widely as potentially valuable tools for crop breeding such as rice (Mackill *et al.,* 1999 and McCouch *et al.*, 1988), wheat and forage species(Jahufer *et al.,* 2003).

According to Krap and Edward (1997), DNA based marker is classified into three categories depending on technique used. Hybridization based DNA markers, arbitrarily primed polymerase chain reaction (PCR)-base markers, and sequence targeted and single locus DNA marker. Restriction Fragment Length Polymorphism (RFLP) is a hybridization based marker in which DNA polymorphism is detected by digesting DNA with restriction enzyme followed by DNA blotting and hybridizations with probes. Sequence Tagged Sites (STS), Sequence Repeat (SSRs), Single Nucleotide Polymorphism (SNPs) markers belongs to sequence targeted and single locus PCR based DNA markers. Microsatellites or SSR are tandem repeats of 1 - 6 nucleotides. For example, $(A)_n$, $(AT)_n$, $(AGT)_n$, $(GATT)_n$, $(GATA)_n$ $(CTACG)_n$, $(TACGAC)_n$, and so on. They are abundant in genome of all organisms. The sequence of unique flanking regions of SSR can be used to design primers and carry out PCR to amplify SSR containing sequences. The polymorphism can be detected by agarose gel electrophoresis if differences are large enough (agarose gels can detect differences greater than 50 base pair). SSRs were first used as markers for use in genetic mapping in humans (Litt and Luty, 1989). SSRs provide a powerful means to link the genetic maps of related species, and since many of them are located within genes of known, any allelic variation present can be utilized to generate perfect markers (Andersen *et al.,* 2003).

Zhuang *et al.* (2004) succeeded to produce a interspecific hybrid from cross between *Cucumis hystrix* and *Cucumis sativas* and investigate the genetic relationship among them using RAPD and SSR markers. PCR based molecular markers RAPD and ISSR evident genetic diversity in the range of 30.8 to 78.6% and 22.7 to 81.2%, respectively among ridge gourd and sponge gourd genotypes (Ravi R. Rathod, 2014). Out of 103 microsatellite markers used for studying the genetic diversity among local

landraces of Luffa species, 56 were found polymorphic, including 38 gSSR and 18 eSSR, respectively. A total of 197 amplification products were obtained. The size of amplified product ranged from 105 to 500 bp. Cucumber-derived SSRs were amplified within *L. acutangula* (68%) (Pandey, 2018). Based on these generated sequences, it was identified 12,932 putative simple sequence repeats (SSRs) and successfully designed 8,523 high-quality SSR primer pairs. Six hundred and forty-one primer pairs were randomly selected to be verified among *Luffa acutangula L.* and L. *cylindrica L.* and their hybrid F1. The result showed that 494 (77.07 %) exhibited successful amplification, of which 201 (40.69 %) revealed polymorphism between S1174 and 93075 (Wu, Hai-BinGong, 2014). By considering the above facts, the present study has been undertaken to identify the hybrids of *Luffa acutangula* and *Luffa cylindrica* through DNA fingerprinting of offspring and parents. The major objectives of the study were: Molecular identification and characterization of parents and hybrids (F_1) and Hybridity detection through SSR marker.

Material and methods

Experimental site and time duration

To confirm hybridity an experiment at molecular level was also carried out during the period from July 2017 to June 2018 at the Biotechnology Laboratory, Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207.

Experimental materials

Seven genotypes of *Luffa acutanguala* and three genotypes of *Luffa cylindrica* were used as experimental materials. Among the materials *of Luffa acutanguala* only BARI Jhinga-1 was collected from Bangladesh Agricultural Research Institute (BARI) and all others from different parts of country. The sources of seeds of the collected genotypes are presented in Table 1.

Leaf sample collection

In order to molecular characterization fresh and young leaf samples were collected at 3-4 leaf stage of each genotype of parents and offspring and used as the source of genomic DNA extraction. Initially, each sample was washed carefully in running tap water and preserved separately. Finally, the samples were brought to the laboratory, wrapped by aluminum foil and stored at -20 °C freezer.

Extraction of genomic DNA

Total genomic DNA was isolated from ridge gourd and sponge gourd following the Phenol: Chloroform: Isoamyl alcohol method and ethanol precipitation technique.

Reagents used in DNA extraction

1. Extraction buffer, pH= 8.0

Compositions of extraction buffer are as follows:

- ✓ 1M Tris-HCl
- ✓ 0.5M EDTA (Ethylene diamine tetra-acetic Acid) (pH= 8.0)
- ✓ 5M NaCl
- ✓ D.H_2O
- ✓ 1% SDS (Sodium Dodecyl Sulphate)

2. Phenol: Chloroform: Isoamyl Alcohol= 25: 24: 1
3. TE buffer, pH=8.0

Compositions of extraction TE buffer are as follows:

- ✓ 1M Tris-HCl
- ✓ 0.5M EDTA
- ✓ D.H_2O

4. Isopropanol
5. 0.3 M Sodium Acetate
6. Absulate (100) ethanol
7. 70% Ethanol
8. RNAase
9. Ethidium Bromide solution.

Confirmation of extracted DNA by electrophoresis

The samples were all in the same concentration in buffer. For each sample, 3µl sample, 3µl dH_2O and 2µl of 2x loading dye (0.255 xylene ethanol, 0.255 bromophenol blue, 30% glycerol and 1mM EDTA) were taken in an eppendorf tube using 0.5-10µl adjustable micropipette. Loading dye was used for monitoring loading and the progress of the electrophoresis and to increase the density of the

sample so that it stayed in the well. The sample was, then loaded into the well of the gel and allowed them to sink to the bottom of the well. The gel was placed in the electrophoresis chamber keeping the gel horizontal and submerged in 1x TBE buffer (running buffer). The gel tank was covered and the electrophoresis power supply was connected and turned on to move DNA from negative to positive (black to red) through the gel. Electrophoresis was carried out at 75volt for about 60 minutes.

Documentation of the DNA samples

The gel was taken from the gel chamber and was placed on an ultraviolet light box (UV trans illuminator) to examine and photographed by a GelCam camera, DNA samples those showing better quality bands were taken for quantification and working solution preparation for next process.

Amplification of SSR markers by PCR

Five SSR primers *viz*. BoG-21, BoG-76, BoG-117, BoG-118, BoG-127 were selected for PCR reaction on 7 ridge gourd, 3 sponge gourd genotypes and their interspecific hybrids to assess their ability of producing polymorphic bands. List of SSR primers, their sequences and GC content were given in Table 2.

PCR amplification and reactions

PCR reactions were performed on each DNA sample. 2x Taq ready Master Mix was used. DNA amplification was performed in oil-free thermal cycler (Esco Technologies swift™ Mini Thermal Cyclers) and Q-cycler, Korea. Preparation of 10.0 µl reaction mixture containing ready mix Taq DNA polymerase is given in Table 3.

Frozen stocks of the PCR reagents i.e., 2x Taq Master Mix, primer and DNA working samples were thawed, spinned and kept on ice for maintaining good quality. DNA samples were pipetted first into PCR tubes compatible with the thermo-cycler used (0.2 ml). A mixture was then prepared by adding PCR reaction mixture, primer, de-ionized water and mixed up well. The tubes were then sealed and placed in a thermo cycler and the thermo cycler was switched on immediately.

PCR amplification thermal profile

DNA amplification was performed in a thermal cycler (Esco Technologies Swift™ Mini Thermal cyclers). Polymerase chain reaction (PCR) technique is used to selectively amplify a specific segment of the total genomic DNA based on the selected SSR primers. SSRs were amplified under the following PCR reaction conditions: Pre-denaturation with 95°C for 4 min; denaturation with 95°C for 40 sec, annealing at 47°C for 33 sec, extension at 72°C for 40 sec, final extension at 72°C for 5min continuing with 33 cycles and finally stored at 4°C.

Electrophoretic separation of the amplified products

PCR products for each sample were confirmed by running it in 1% agarose gel containing 0.75µl ethidium bromide in 1X TBE buffer at 75 Volt for 1 hour. 5 µl Loading dye was added to the PCR product and spinned them well. Then loaded them in the wells and 100 bp DNA ladder (Bioneer) was also placed in both left side of the gel. Under ultra-violet light on a trans-illuminator SSR bands were observed.

Documentation of PCR amplified DNA products

The gel was taken out carefully from the gel chamber and was placed on high performance ultra-violet light box (UV trans-illuminator) of gel documentation for checking the DNA band and photographed by a Gel Cam Polaroid camera. Since SSR markers are co dominant hence, each band represented the phenotype at a single allelic locus (Williams *at el.,* 1990). One molecular weight marker, 100bp (BIONEER, Cat. No. M-1070-Bio Basic, Canada) DNA ladder was used to estimate the size of the amplification products by comparing the distance traveled by each fragment with known sized fragments of molecular weight markers. All distinct bands or fragments were thereby given identification numbers according to their band and size scored visually on the basis of their presence (1) or absence (0), separately for each individual and each primer.

This was done separately for individual fragments and primers. The band size for each markers was scored. The scores obtained for the SSR primers were then used to assess the polymorphism of parents and to test their F_1 hybrids.

Result and discussion

DNA extraction and confirmation

Genomic DNA was extracted in the compatible genotypes and the progeny of compatible crosses by the CTAB method with minor modification. RNA sharing was removed by applying RNAse treatment. Finally, the purified DNA was stored at - 20°C freezer for further use.The extracted genomic DNA of 17 samples were loaded on 1% agarose gel for conformation and quantification of DNA. Most of all the samples showed clear DNA band in well (Fig. 1) but some smear was observed.

The genomic DNA of each sample was diluted on the basis of concentration to make the working DNA sample for PCR amplification.

Table 1. Name and sources of genotypes.

Sl. No	Name of the Genotypes	Collected from
01	BARI Jhinga-1 (*Luffa acutanguala*)	Bangladesh Agriculture Research Institute, Gazipur.
02	RIG-19\2 (*Luffa acutanguala*)	Savar, Dhaka
03	RIG47\2 (*Luffa acutanguala*)	Savar, Dhaka
04	RIG 83\2 (*Luffa acutanguala*)	Savar, Dhaka
05	KRG 02 (*Luffa acutanguala*)	Savar, Dhaka
06	KRG 04 (*Luffa acutanguala*)	Savar, Dhaka
07	Purbachal (*Luffa acutanguala*)	Savar, Dhaka
08	Bogra local (*Luffa cylindrica*)	Bogra District
09	Faridpur local (*Luffa cylindrica*)	Faridpur District
10	Lalmonirhat local (*Luffa cylindrica*)	Lalmonirhat District

Table 2. List of SSR primers, their sequences and GC content.

Sl. No.	Name of SSR primer (Trade Name)	Sequences of the primer (5'-3')	% of (G+C) content	Annealing temperature (0°C)
1	BoG 21	For. GTATATGGGTCGTATTGGGA	45	58.3
		Rev. TCAGTGAAATCTGACCTCAA	40	
2	BoG 76	For. TTCTTTTCATGGGATAGAGC	40	58.3
		Rev. GCAACAAAAGAGATAAGCCA	40	
3	BoG 117	For. CTCGTTCACGTTCTTCTAAC	50	59.0
		Rev. GTTTATTTAGGGGTTGCCTT	40	
4	BoG 118	For. GGAAGTTCTCTTCCTCGATT	46	58.6
		Rev. TTCTCCTGACTCTCACCCTA	40	
5	BoG 127	For. CTTTGAGAGAGAAGTGTGGG	45	58.6
		Rev. GCCTTACATGTGAACAAACA	40	

Hybridity test through molecular marker

To achieve this objective huge number of molecular marker has to be screened between two parents of a specific hybrid. SSR primer is the best for the study because it will give co-dominant band among the studied genotypes. Polymorphism can be easily identified from the DNA amplification pattern. DNA fragment amplified by the molecular marker present in male parent and hybrid offspring and it was absent in female parent is important indication of hybridity test. It proved that, this portion of genome has come from male parent in hybrid variety. Hence, it is amplified only in male parent and in hybrid offspring.

Normally hybrid seed is collected from the female parent. If any DNA amplification present in female parent and hybrid offspring but not in male parent than it is indicator of female genome. Hence, this type of DNA band is not suitable for hybridity test. It should be discarded for hybridity analysis.

Table 3. Reaction mixture composition for PCR for each genotype.

Reagents	Amount (µl)
2x Taq Master Mix	5.0
SSR Forward primer	1.0
SSR Reverse primer	1.0
De- ionized water	1.5
Sample DNA	1.5
Total reaction volume	10.0

Table 4. DNA amplification status of the hybrid Bogra×RIG47-2 and its corresponding parents.

Sl. No.	Primer name	Primer sequence (5'-3')	DNA amplification			Marker type
			Female Parent	Male Parent	Hybrid	
1	BoG 21	For. GTATATGGGTCGTATTGGGA Rev. TCAGTGAAATCTGACCTCAA	Amplified	Amplified	Amplified	Not suitable for hybridity test
2	BoG 76	For. TTCTTTTCATGGGATAGAGC Rev. GCAACAAAAGAGATAAGCCA	Not Amplified	Amplified	Amplified	Suitable for hybridity test
3	BoG 117	For. CTCGTTCACGTTCTTCTAAC Rev. GTTTATTTAGGGGTTGCCTT	Not Amplified	Amplified	Amplified	Suitable for hybridity test
4	BoG 118	For. GGAAGTTCTCTTCCTCGATT Rev. TTCTCCTGACTCTCACCCTA	Not Amplified	Not Amplified	Not Amplified	Not suitable for hybridity test
5	BoG 127	For. CTTTGAGAGAGAAGTGTGGG Rev. GCCTTACATGTGAACAAACA	Not Amplified	Amplified	Amplified	Suitable for hybridity test

The above discussion reveals that, for hybridity test through molecular marker need specific DNA amplification which is present in male and hybrid offspring but not in female parent. By applying this principle this research work has been done in four different hybrid and its corresponding parents. The individual result is given below. It reveals that, four primers viz BoG 21, BoG 76, BoG 117 and BoG 127 showed DNA amplification in three hybrids and their corresponding parents.

Table 5. DNA amplification status of the hybrid Bogra×BARI Jhinga-1 and its corresponding parents.

Sl No	Primer name	Primer sequence (5'-3')	DNA amplification			Marker type
			Female Parents	Male Parents	Hybrid	
1	BoG 21	For. GTATATGGGTCGTATTGGGA Rev. TCAGTGAAATCTGACCTCAA	Not Amplified	Amplified	Amplified	Suitable for hybridity test
2	BoG 76	For. TTCTTTTCATGGGATAGAGC Rev. GCAACAAAAGAGATAAGCCA	Not Amplified	Amplified	Amplified	Suitable for hybridity test
3	BoG 117	For. CTCGTTCACGTTCTTCTAAC Rev. GTTTATTTAGGGGTTGCCTT	Not Amplified	Amplified	Amplified	Suitable for hybridity test
4	BoG 118	For. GGAAGTTCTCTTCCTCGATT Rev. TTCTCCTGACTCTCACCCTA	Not Amplified	Not Amplified	Not Amplified	Not suitable for hybridity test
5	BoG 127	For. CTTTGAGAGAGAAGTGTGGG Rev. GCCTTACATGTGAACAAACA	Not Amplified	Amplified	Amplified	Suitable for hybridity test

The molecular marker BoG 21 produced 550 bp DNA fragement in both male (RIG47-2) and hybrid (Bogra×RIG47-2**)** and female (Bogra) produce 350 bp DNA band. In case of Bogra×BARI Jhinga-1 male (BARI Jhinga-1**)** and hybrid (Bogra×BARI Jhinga-1) produced 250 bp but female (Bogra) did not produce any band (Fig. 2). In case of primer- BoG 76 the hybrid (Bogra× RIG 47-2) produced 450 bp DNA band and male (RIG 47-2) produced same band but female (Bogra) did not produce any band. Here, Male and hybrids giving band and we concluded that it may be hybrid. Another hybrid (Bogra×BARI Jhinga-1) and male (BARI Jhinga-1) produced 200 bp but female (Bogra) did not produce any band. So we concluded that it may be hybrid as the hybrids produce the same band of male parent (Fig.3).

Table 6. DNA amplification Status of the hybrid Bogra×Purbachal and its corresponding parents.

Sl No	Primer name	Primer sequence (5'-3')	DNA amplification			Marker type
			Female Parents	Male Parents	Hybrid	
1	BoG 21	For. GTATATGGGTCGTATTGGGA Rev. TCAGTGAAATCTGACCTCAA	Amplified	Not Amplified	Amplified	Not suitable for hybridity test
2	BoG 76	For. TTCTTTTCATGGGATAGAGC Rev. GCAACAAAAGAGATAAGCCA	Amplified	Not Amplified	Amplified	Not suitable for hybridity test
3	BoG 117	For. CTCGTTCACGTTCTTCTAAC Rev. GTTTATTTAGGGGTTGCCTT	Amplified	Not Amplified	Amplified	Not suitable for hybridity test
4	BoG 118	For. GGAAGTTCTCTTCCTCGATT Rev. TTCTCCTGACTCTCACCCTA	Not Amplified	Not Amplified	Not Amplified	Not suitable for hybridity test
5	BoG 127	For. CTTTGAGAGAGAAGTGTGGG Rev. GCCTTACATGTGAACAAACA	Amplified	Not Amplified	Amplified	Not suitable for hybridity test

Table 7. DNA amplification Status of the hybrid Faridpur×KRG 02 and its corresponding parents.

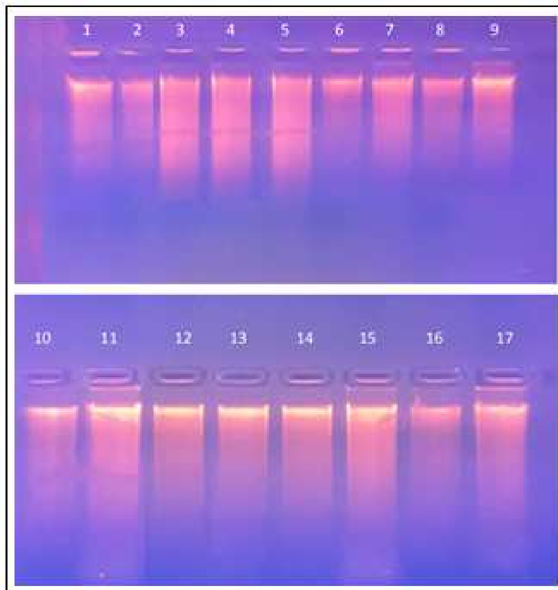
Sl No	Primer name	Primer sequence (5'-3')	DNA amplification			Marker type
			Female Parents	Male Parents	Hybrid	
1	BoG 21	For. GTATATGGGTCGTATTGGGA Rev. TCAGTGAAATCTGACCTCAA	Amplified	Amplified	Amplified	Suitable for hybridity test
2	BoG 76	For. TTCTTTTCATGGGATAGAGC Rev. GCAACAAAAGAGATAAGCCA	Amplified	Amplified	Amplified	Suitable for hybridity test
3	BoG 117	For. CTCGTTCACGTTCTTCTAAC Rev. GTTTATTTAGGGGTTGCCTT	Amplified	Amplified	Amplified	Suitable for hybridity test
4	BoG 118	For. GGAAGTTCTCTTCCTCGATT Rev. TTCTCCTGACTCTCACCCTA	Not Amplified	Not Amplified	Not Amplified	Not suitable for hybridity test
5	BoG 127	For. CTTTGAGAGAGAAGTGTGGG Rev. GCCTTACATGTGAACAAACA	Amplified	Amplified	Amplified	Suitable for hybridity test

The hybrid Faridpur×KRG 02 produces monomorphic band when amplified with primer BoG 117. When monomorphic DNA band is present in all genotypes, it indicate that the primer is not suitable for hybridity test. Thus, this primer was not suitable for hybridity test for the hybrid Faridpur×KRG 02. No polymorphism also occured in the parent of the hybrid by the primer BoG 117. Hence, it can not be used for the hybridity detection of the hybrid Faridpur×KRG 02 (Fig.4).

Table 8. Summary of DNA amplification pattern in F_1 hybrid.

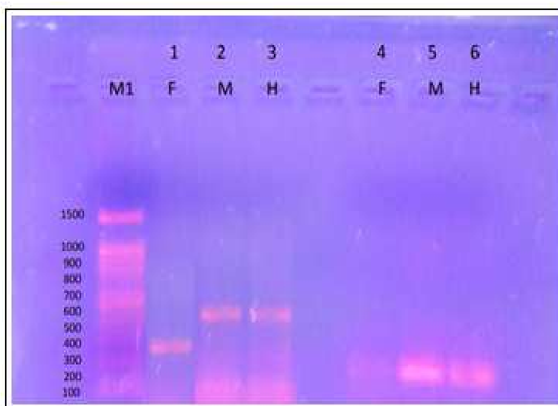
Sl. No.	Primer name	Primer sequence (5'-3')	(G+C)%	No. of DNA band(s)	Number of Hybrid	Band size ranges (bp)
1	BoG 21	For. GTATATGGGTCGTATTGGGA Rev. TCAGTGAAATCTGACCTCAA	46	1	Bogra×RIG47-2 Bogra×BARI Jhinga-1	550 250
2	BoG 76	For. TTCTTTTCATGGGATAGAGC Rev. GCAACAAAAGAGATAAGCCA	49	1	Bogra×RIG47-2 Bogra×BARI Jhinga-1	450 200
3	BoG 117	For. CTCGTTCACGTTCTTCTAAC Rev. GTTTATTTAGGGGTTGCCTT	50	1	Faridpur×KRG 02	400
4	BoG 118	For. GGAAGTTCTCTTCCTCGATT Rev. TTCTCCTGACTCTCACCCTA	43	1	----	---
5	BoG 127	For. CTTTGAGAGAGAAGTGTGGG Rev. GCCTTACATGTGAACAAACA	44	1	Bogra×BARI Jhinga-1	450

It is discussed in the principle of hybridity test that, if a primer produces DNA band in the male parent but not in the female parent and same band in hybrid genome this type of amplification can be used for hybridity test. Fifty percent (50%) of male genome was transferred to hybrid variety through pollen grain. Hence, the DNA fragment which was present in male and hybrid genotype but not in female genotype is the indicator of hybrid variety.



Lane 1 =Bogra, Lane 2 = BARI Jhinga-1, Lane 3 =Bogra× BARI Jhinga-1, Lane 4 = RIG-19-2, Lane 5 = Bogra×RIG 19-2, Lane 6 = RIG 47-2, Lane 7 = Bogra×RIG 47-2 , Lane 8 =KRG 02, Lane 9 = Bogra×KRG 02 , Lane 10 = Purbachal, Lane 11= Bogra×Purbachal, Lane 12=Faridpur, Lane 13=Faridpur× RIG 19-2, Lane 14=Faridpur×KRG 02, Lane 15= KRG 02× Bogra, Lane 16 = RIG-83-2 Lane17= KRG 02.

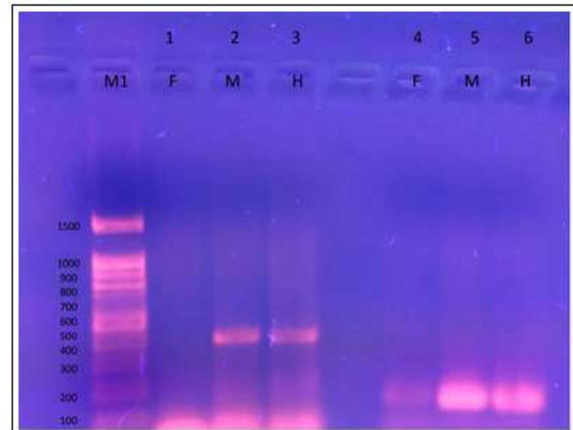
Fig. 1. Genomic DNA from different genotypes of ridge gourd and sponge gourd and their hybrid.



M1=100bp DNA ladder, Lane1=Bogra, Lane2=RIG 47-2, Lane3=Bogra× RIG 47-2, Lane4=Bogra, Lane5=BARI Jhinga-1, Lane6= Hybrid (Bogra×BARI Jhinga-1).

Fig. 2. The SSR primer BoG 21 was used for hybrid identification.

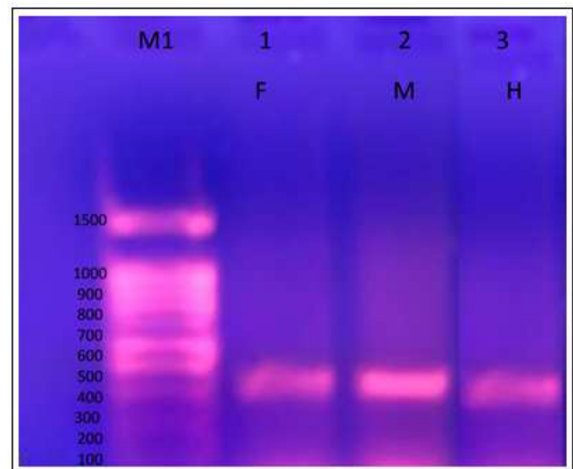
It is interesting to note that, the primer BoG 21 produced polymorphic DNA fragment among the parents of the hybrid Bogra×RIG47-2 (Fig. 2) and the primer gave 550bp DNA band in male parent and hybrid. BoG 76 produced polymorphic DNA fragment among the parent of the hybrid Bogra×BARI Jhinga-1 (Fig. 3).



M = 100 bp DNA ladder, Lane 1= Bogra , Lane 2 = RIG47-2, Lane 3 = hybrid (Bogra×RIG47-2) Lane 4= Bogra , Lane 5= BARI, Lane 6 = hybrid (Bogra×BARI Jhinga-1).

Fig. 3. PCR amplification with SSR primer BoG 76.

The primer gave 200bp DNA band in male parent and hybrid and it was absent in female parent. The hybrid variety also showed the male parent type of DNA band. The replicated trial also showed the same pattern of DNA amplification. Hence, this type of primer (Molecular marker) can use for large scale screening of hybridity test.



M1 = 100 bp DNA ladder, Lane 1= Faridpur, Lane 2 = KRG 02, Lane 3 = Hybrid (Faridpur×KRG 02).

Fig. 4. PCR amplification with SSR primer BoG 117 in case of hybrid Faridpur×KRG 02.

Polymorphism survey, band size and banding pattern of hybrid of ridge gourd and sponge gourd and their corresponding parents

Five molecular markers *viz* BoG 21, BoG 76, BoG 117, BoG 118 and BoG 127 were used for hybridity detection in four hybrids those getting from crosses between of ridge gourd and sponge gourd. The DNA

amplification status of each primer and hybrid are given in Table 4 to 8.

Conclusion

It is need to conduct studies to evaluate the molecular analysis for breeding and conservation purposes. This research investigation presented mainly the hybridity test of that progenies which were obtained by crossing between ridge gourd and sponge gourd through molecular marker. Five different SSR primers (BoG 21, BoG 76, BoG 117, BoG 118, BoG 127) were used to establish polymorphism among the parents (BARI Jhinga-1, Bogra, Faridpur, RIG 19-2 etc.) of each hybrid. The identified polymorphic primers were used to screen the hybrid variety together with corresponding parents. It was found that three primers viz. BoG 21, BoG 76, and BoG 117 showed polymorphism in the parents of hybrids. These primers were used for the detection of hybrid variety. It was noted that, a 550bp DNA fragment was amplified in male parent and hybrid of Bogra×RIG 47-2 which was absent in female genotype. 200 bp DNA fragment was amplified in male parent and hybrid in case of Bogra×BARI Jhinga-1. This DNA band can be used for large scale screening of that specific hybrid variety for seed purity in commercial purpose. The parents of other hybrid showed monomorphic DNA band and some of the SSR primers (BoG 118 and BoG 127) were not amplified in any ridge gourd or sponge gourd genotype under investigation.

The idea would be accepted by policy makers and govt. of authority to ensure quality seed. The farmers will not be cheated by the seed companies and they will be able to get pure seeds of hybrid variety. The commercial aspects of new innovation may be viable for all the hybrid seed companies of Bangladesh.

Recommendations

Large number of varieties would be needed to study and higher number of primers would be necessary to identify the hybrids and their corresponding parents. Details survey work should be conducted using more molecular markers for obtaining polymorphism among the parents. Other molecular markers such as ISSR, AFLP, etc. should be used for further confirmation.

The results indicate that the present study might be used as a guideline for further study and the following points might be considered for developing the better quality ridge gourd and sponge gourd varieties:

Abbreviation

SSRs=Simple Sequence Repeats.

BARI = Bangladesh Agriculture Research Institute.

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