INTERSPECIFIC CROSS COMPATIBILITY STUDY BETWEEN *Luffa acutangula* AND *Luffa cylindrica* AND MOLECULAR ASSESSMENT OF HYBRIDITY IN F_1 GENERATION

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CERTIFICATE

This is to certify that the thesis entitled ***"INTERSPECIFIC CROSS COMPATIBILITY STUDY BETWEEN Luffa acutangula AND Luffa cylindrica AND MOLECULAR ASSESSMENT OF HYBRIDITY IN F_1 GENERATION"*** *submitted to the Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (MS) in BIOTECHNOLOGY,*** *embodies the result of a piece of bonafide research work carried out by* ***MD. SHAMIM AHMED,*** *Registration No.* ***12-05107*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed during the course of this investigation has been duly acknowledged and style of this thesis have been approved and recommended for submission.

Dated: June, 2018
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ABBREVIATIONS AND ACRONYMS

FULL WORD	ABBREVIATION
Angstrom	A°
Amplified Fragment Length Polymorphism	AFLP
And others (at elli)	*et al.*
Bangladesh Agricultural Research Institute	BARI
Base pair	Bp
Centimeter	Cm
Cetyl Trimethyl Ammonium Bromide	CTAB
Continued	Cont'd
Double distilled water	dd H_2O
Degree celsius	^0C
Deoxyribonucleic acid	DNA
Deionized water	dH_2O
Diversity Arrays Technology	DArT
Ethylene Diamine Tetra Acetic Acid	EDTA
Etcetera	etc.
Ethidium Bromide	Et-Br
Government	Govt.
Gram	G
Grow Out Test	GOT
Gram per Liter	g/L
Genetic diversity index	GDI
Inter Simple Sequence Repeats	ISSRs
Marker assistant breeding	MAS
Microliter	µl
Micro mole	µM
Milliliter	ml
Namely	*viz.*
Negative logarithm of hydrogen ion concentration (-log[H+])	pH
Percent	%

ABBREVIATIONS (Cont'd)

FULL WORD	ABBREVIATION
Polymorphic Information Content	PIC
Polymerase Chain Reaction	PCR
Random Amplified Polymorphic DNA	RAPD
Research and Development	R&D
Restriction Site Associated DNA Markers	RAD
Restriction Fragment Length Polymorphism	RFLP
Rotation per minute	Rpm
Seed Certification Agency	SCA
Short Tandem Repeat	STR
Simple Sequence Length Polymorphism	SSLP
Single Feature Polymorphism	SFP
Single Nucleotide Polymorphism	SNP
Simple Sequence Repeat	SSR
Sodium chloride	NaCl
Sodium Dodecyl Sulphate	SDS
Species	spp.
Luffa	*L.*
Thermophilus aquaticus	Taq
Tris Boric Acid EDTA	TBE
Tris-EDTA	TE
tons	T
Unweighted Pair Group of Arithmetic Mean	UPGMA
Ultra Violet	UV
Volt	V

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The Author

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ABSTRACT

An experiment was carried out to study of the cross compatibility between ridge gourd and sponge gourd and assess hybridity in F_1 generation through SSR marker. Field experiment was conducted at the farm of Sher-e-Bangla Agricultural University and laboratory experiment was conducted at Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh during the period of March 2017 to June 2018. Seven ridge gourd and three sponge gourd genotypes were used as parents. Most of the crosses were compatible such as Bogra×BARI Jhinga-1, Bogra×RIG 47-2, Faridpur×RIG 19-2 etc. and some crosses are incompatible such as Lalmonirhat×BARI Jhinga-1, Lalmonirhat×RIG 19-2 etc. Cross compatibility was maximum 75% in Bogra×BARI Jhinga-1. Fruit size and weight of the compatible crosses were intermediate between ridge gourd and sponge gourd. The interspecific hybrids produced fruits that have less number of seeds with less fiber density than that of the *L. cylindrica* entries. 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 hybrids. 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. Again, 200 bp DNA fragment was amplified in male parent and Bogra×BARI Jhinga-1 hybrid. 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 genotypes.

CHAPTER I

INTRODUCTION

Ridge gourd (*Luffa acutangula* L., 2n=26), popularly known as 'Jhinga' is one of the most important cucurbitaceous vegetables in Bangladesh available during March to August. The fruit surface is ridged and dark green in color with white pulp inside. It is extremely rich in dietary fibre and enriched with all the vital elements that include vitamin-C, zinc, iron, riboflavin, magnesium, thiamine and traces of another minerals (Yawalkar, 1985). The ridge gourd has some amazing health benefits and medicinal properties. Ridge gourd contains good amount of cellulose and high in water content that helps in release from constipation. It contains insulin like peptides and alkaloids that helps to reduce the sugar levels in the blood and urine. It is rich in beta-carotene which is good for enhancing eye sight. Ridge gourd is an effective blood purifier. It boosts up and nourishes the liver health and protects the liver from alcohol intoxication. Ridge gourd juice is used to rectify jaundice and to strengthen immune system against any infection. It is good for skin care and helps to manage acidity as well as ulcers. It is well known as a cooling agent and aids in handling burning sensation during urination. A glycoprotein isolated from the seeds of *L. acutangula* was found to be immunologically distinct from proteins isolated from other members of the cucurbitaceae family (Yeung *et al.,* 1991). The luffaculin, a protein isolated from its seed, exhibited abortifacient, antitumor, ribosome inactivating and immune modulatory activities (Ng *et al.,* 1992).

So far, more than 106 local landraces of *L. acutangula* have been reported in Bangladesh (Rabbani, 2007). The average yield of the crop in Bangladesh is about 3.83 mt per acre in 2017-2018 (BBS, 2018) indicating low yield performances of the cultivars.

Luffa cylindrica is a herbaceous, monoecious vine, also a cucurbitaceous vegetable in Bangladesh. It is a versatile vegetable that can be used as a household cleaning product, industrial material and medicine and possesses anti-human immune

deficiency virus activity. *Luffa cylindrica* has some special characteristics over *Luffa acutangula* such as better fiber density, adaptable to wide range of climatic and soil conditions etc. Furthermore, better resistance to fruit fly was observed among the *L. cylindrica* (Reyes, 1994). Thus *Luffa cylindrica* having more tolerance to biotic and abiotic stress compared to *Luffa acutangula* such as more drought tolerant, more weed tolerant etc. If the *Luffa acutangula and Luffa cylindrica* become cross compatible then we can easily transfer the gene between them and can improve the crop quality.

Interspecific hybridization allows a transfer of genes of interest between different species especially those involved in disease resistance (Bosland and Votava, 2000), allowing breeders to develop genetically superior genotypes. As a general rule, the closer the species involved in the cross will be genetically the easier it to breed hybrids and the more fertile the progenies (Singh 2002). For the future crop improvement program cross compatibility between the species might contribute to develop heterotic hybrid.

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. Moreover, we can use

molecular technique at the early vegetative stage of crop such as leaf stage, not necessary to wait for fruit harvest.

A molecular marker is a gene or DNA sequence with a known location on a chromosome that can be used to identify individuals or species. A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism, SNP), or a long one, like minisatellites. Markers can exhibit two modes of inheritance, i.e. dominant/recessive or co-dominant. If the genetic pattern of homozygotes can be distinguished from that of heterozygotes, then a marker is said to be co-dominant. Generally co-dominant markers are more informative than the dominant markers. (Manikanda, 2012).

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).

Polymorphism or genetic variation among the parents is pre-requisite for crossing test through molecular marker. Variation at DNA level is used as an indicator for hybridity. Hence polymorphism study among the parent is essential. SSR primers are used for polymorphism study. After establishment of polymorphism among the parents using SSR markers those specific SSR primers can be used for screening of the hybrid as well as its respective parents. By considering the above facts, the present study has been undertaken to study the cross compatibility between ridge gourd and sponge gourd varieties and molecular identification of parents and hybrids (F_1). So, the major objectives of the study are as follows:

OBJECTIVES:

1. Morphological characterization of *Luffa acutangula* and *Luffa cylindrica* genotypes

2. To study the cross compatibility between *Luffa acutangula* and *Luffa cylindrica* genotypes

3. To estimate the success rate of interspecific cross

4. Phenotypic characterization of hybrids (F_1)

5. Molecular identification and characterization of parents and hybrids (F_1).

CHAPTER II

REVIEW OF LITERATURE

The present study was conducted to study of the cross compatibility between ridge gourd and sponge gourd and identify their hybrids through molecular markers. Some of the research works relating to this are reviewed in this chapter.

2.1 Floral morphology and biology of Ridge gourd and Sponge gourd genotypes

Rashid (1993) noted that the color of the flowers of ridge gourd is yellow in color and slightly perfumed. Calyx contains five united sepals in male flower but separated in female flower. Stamens are three in number and among those two are larger in size than the third. The number of node at which first male and female flowers opened ranges from 20^{th} to 30^{th}. Rashid (1993) also noticed that sponge gourd is monoecious which bears staminate and pistillate flowers in the same plant. The flowers were large, short or long pedicellate and yellow in color. Calyx comprised of five united but deeply lobed sepals in male but separated in female flowers. Corolla consisted of five separate bell shaped petals. Stamens were five in number and anthers are free. Ovary was long, filiform and covered with soft hairs. The length of ridge gourd fruit varied from 15 cm to 40 cm.

Bhandari (1979) studied on floral biology in ridge gourd and reported that ridge gourd staminate flowers are racemose type of inflorescence and pistilate flowers are solitary. The flowers are yellow or white in color and pedicellate. Calyx comprises five united but deeply lobed sepals. Corolla consists of five united hell shaped petals in imbricate aestivation. The number of stamens are three and anthers are free. Ovary is inferior, ribbed, angled and tricarpellary. Stigmas are three in number as well as forked. Bhandari (1979) also reported that anthesis starts from 4 p.m. and continues till 6 p.m. It is followed by anther dehiscence which commences 10-15 minutes before the anthesis and complete half and hour after anthesis. He also noticed that stigma became receptive two to seven hours before anthesis and it continues 36 hours after anthesis. However, stigma was perfectly receptive on the day of anthesis.

Choudhury (1967) reported that common varieties of ridge gourd are monoecious like bottle gourd, pumpkin and melons. They bear the male and female flowers separately on the same plant.

Arora et al. 1983 observed in sponge gourd that the node number of first female flowers opened ranges from 8^{th} to 20^{th}. He also reported in sponge gourd that days to first male and female flowering ranged from 56 to 118 days and 61 to 125 days respectively.

Krisna et al. 1989 noted in ridge gourd that the number of node at which first male and female flowers opened was an average of 7^{th} to 16^{th}. The range of total number of fruits per plant of 11 varieties of ridge gourd was 26 to 86.

In both sponge and ridge gourd, Chauhan (1989) observed that flowering started within 60 to 80 days after the sowing of seed and the flowers of both sponge and ridge gourd opened from 4.30 am to 6 am. He also noticed in sponge gourd that the fruit number per vine varied from 25 to 30.

Akand 1993, observed in ridge gourd that the first male flower opened within 42 to 46 days, and first female flower opened within 48 to 52 days, while for hybrid it ranged from 40 to 45 days and 43 to 51 days for male and female flower anthesis respectively. The mean performance of fruits per plant of 20 ridge gourd hybrids and their parents and found that the total number of fruits per plant ranged from 5.22 to 6.11.

Latif 1993 noted in ridge gourd that the number of days to male flower opening in five parental lines and for their hybrids ranged from 46 to 49 and 46 to 51 days and that for female flower it ranged from 51 to 54 and 50 to 55 days respectively. The ranges of total number of fruits per plant of 5 ridge gourd inbreed lines and their 10 F_1 hybrids were 17.43 to 25.35.

Anthesis in ridge gourd was between 16.00 and 19.30 h. Anther dehiscence preceded flower opening and took 1.5-2.5 h to complete. Pollen viability was high, ranging from 94% to 98%. Pollen grain size varied considerably within and among varieties. Stigma receptivity, judging by fruit set, reached a maximum (76%) at anthesis; fruit set was 19% by 12 h after anthesis (Deshpande, 1980).

Joshi and Singh (1967) found that in both sponge and ridge gourd the stigma became receptive 7 hours before anthesis and remained active for 40 hours after anthesis.

Murty, (1989) reported that the fruits of sponge gourd were nearly cylindrical normally with light furrows or stripes but not ribbed, green in colour, 30-60 cm long. Rashid (1993) reported that the fruits of ridge gourd are tubular shaped or club shaped, deep green in colour and ten ridges are present on the surface of fruit.

2.2 Interspecific cross between Ridge gourd and Sponge gourd

Begum (1998) observed Cross compatibility in Japanese and indigenous sponge gourd (*Luffa cylindrical L.*) genotypes and hybrid offspring gave higher yield (8.0 kg/plant) than local cultivars (4.0 kg/plant).

In an attempt to develop suitable cultivars for sponge production and processing, a preliminary evaluation was conducted to explore the potentials of interspecific hybrids of *Luffa spp*. Five hybrids between *L. cylindrica* (smooth) and *L. acutangula* (ridged) together with an OP cultivar of *L. acutangula*, four lines and an F1 hybrid of *L. cylindrica* were evaluated in observational plots at IPB, UPLB. The interspecific hybrids had semi-ridged fruits that are very bitter. The mature brown fruits of these hybrids can easily be processed unlike that of the *L. acutangula* entry Rdp-1 (Reyes, 1991).

Four varieties of *L. cylindrica* were crossed with one variety of *L. acutangula*. Only the crosses with varieties T28 and T21 of *L. cylindrica* as female parents survived. The morphological features of the F_1 plants are shown to be generally intermediate between those of the parents (Pathak and Sing, 1949).

Experimental amphidiploids were induced by colchicine treatment of the F_1 hybrids of *Luffa acutangula x L. graveolens*. These amphidiploids showed gigantism over the diploid F_1 hybrids in all morphological features recorded, but fruit setting did not occur in spite of artificial pollination of the female flowers (Bithi dutt, 2014).

The interspecific hybrids produced sponges with much better fiber density than that of the *L. cylindrica* entries. Furthermore, higher yield in terms of number of fruits per

plant and better resistance to fruit fly were observed among the interspecific hybrids than either the *L. cylindrica* or *L. acutangula* entries (Reyes, 1991).

2.3 The concept of molecular marker

Molecular markers are reliable tools to characterize the DNA profiling of plant genotypes to study the genetic diversity. According to Datta *et al*., (2011) molecular markers are specific fragments of DNA that can be identified within the whole genome. Molecular markers are found at specific locations of a genome.

Molecular markers have been found to be more dependable than the phenotypic observations for estimating the variations and in the assessment of the genetic stability (Leroy *et al.*, 2000) and 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. The molecular marker techniques are diverse and variable in principle, application and amount of polymorphism observed and in time requirement. Molecular markers present an efficient tool for fingerprinting of cultivars, and assessment of genetic similarities and relationships (Vilanova *et al.*, 2012).

With the advent of molecular biology techniques, molecular markers are effective because they identify the abundance of genetic linkage between identifiable locations within a chromosome and are able to be repeated for verification. A molecular marker is a DNA sequence that can be readily detected and whose inheritance can easily be estimated. Amin *et al*., (2010) stated that, the use of molecular markers is based on naturally occurring DNA polymorphism, which forms the basis for designing strategies to exploit for applied purposes.

Molecular markers are able to identify particular locations on a chromosome, allowing creation of physical maps. Molecular marker can identify many alleles within an organism for a particular trait (bi allelic or poly allelic).

Molecular marker can identify small changes within the mapping population enabling distinction between a mapping species, allowing for segregation of traits and identity. Some studies which were conducted during the last decade of the 20th century reported numerous DNA markers that have been utilized in plant breeding programs. Apart from the application of molecular markers in the construction of linkage maps,

they have numerous applications in plant breeding such as assessing the genetic variations within cultivars and germplasms (Henry,1997).

The most interesting application of molecular markers is marker-assisted selection (MAS). They have proved to be excellent tools for assessment of genetic diversity in a wide range of plant species (Madhumati, 2014).

Suitable DNA markers should be polymorphic in the DNA level and can be expressed in all tissues, organs, and various developmental stages. The molecular approach for identification of plant genotypes seems to be more effective as it allows direct access to the hereditary material unlike the morphological markers (Paterson *et al.,* 1991).

Molecular markers can be used for molecular characterization and detecting genetic variation and relationship of plants. These markers can detect the variation that arises from deletion, duplication, inversion, and/or insertion in the chromosomes. Such markers themselves do not affect the phenotype of the traits of interest because they are located only near or linked to genes controlling the traits (Mondini *et al.,* 2009).

Amin *et al.* (2010) reported the use of molecular markers to identify the vegetable crop varieties like tomato, potato, onion, garlic and related species and linked to major disease resistance in tomato like *Meloidogyne incognita* and tomato mosaic virus. These markers are selectively neutral because they are usually located in non-coding regions of DNA. Usually located in non-coding regions of DNA. Unlike morphological and biochemical markers, DNA markers are practically unlimited in number and are not affected by environmental factors. Apart from the use of DNA markers in construction of linkage maps, they have numerous applications in plant breeding such as assessing the level of genetic diversity within cultivars and fingerprinting the germplasms. 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. Of these, RFLP and micro satellites are co-dominant markers, while RAPD and AFLP markers are largely dominant markers.

2.4 Microsatellite or Simple Sequence Repeats (SSRs) marker

Microsatellites (SSRs) are short tandem repeats of simple (1–6) nucleotide. Their value for genetic analysis lies in their multi-allelism, co-dominant inheritance, relative abundance, genome coverage and suitability for high-throughput PCR-based platforms. They can serve as highly informative genetic markers, and in conjunction with the use of polymerase chain reaction (PCR) technology enable the detection of length variation (Powell, 1996).

Microsatellite primers developed for one species can be used to detect polymorphism at homologous sites in related species. They have become one of the most useful molecular marker systems in plant breeding. The development of SSR markers from genomic libraries is expensive and inefficient (Squirrell *et al.*, 2003).

It was long assumed that SSRs were primarily associated with non-coding DNA, but it has now become clear that they are also abundant in the single and low-copy fraction of the genome (Toth *et al.,* 2000). SSRs are commonly referred to as "genic SSRs" or "EST-SSRs" and are present in 1 to 5% of the expressed plant DNA sequence (Varshney *et al.*, 2005).

SSR markers are non-isotype based precise PCR technology. Stretches of DNA, consisting of randomly repeating small nucleotide units and conserved regions flanking the repeats are suitable for designing PCR primer pairs. Used for amplifying the intervening repeat loci were first referred to as Microsatellites by Litt and Luty (1989) and later as Simple Sequence Repeats (SSRs) by Jacob *et al*.(1991).

Smith *et al.* (1997) made a comparison of SSR with data from RFLP and pedigree in maize. They stated that SSR revealed co-dominantly inherited multi-allelic product of loci that can be readily mapped. SSR profiles can be interpreted genetically without the need to repeatedly map amplified bands to marker loci in the different populations. They anticipated that SSR profiling will replace RFLP and PCR based arbitrary primer methods.

On the other hand, 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 exploited to generate perfect markers (Andersen *et al.,* 2003).

2.5 Microsatellite or Simple Sequence Repeats (SSRs) marker used in Ridge gourd and Sponge gourd

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).

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.

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, *et al.,* 2014).

By using DNA based hybridity test Mahbuba (2017) using SSR primers and identified four eggplant hybrids named MEH5003, MEH5006, MEH5009, MEH5012 in the laboratory of Biotechnology Department of Sher-e-Bangla Agricultural University.

CHAPTER III

MATERIALS AND METHODS

The materials and methods used in the experiment are focused on this chapter. The detail of the methodology followed in the study has been described as follows:

3.1 Experimental site and time duration

The experiment was conducted in both field and laboratory

Experiment in the field condition

The experiment was conducted at the central farm, Sher-e-Bangla Agricultural University, Dhaka-1207 (90°33' E longitude and 23°77' N latitude) under AEZ 28 (Madhupur Tract).

Parental materials of *Luffa acutangula* and *Luffa cylindrica* were sown and interspecific cross compatibility study was performed from March 2017 to August 2017. Seeds are collected from the crossing fruit and sown in next season for studying the characteristics of offspring from March 2018 to June, 2018.

Experiment in the laboratory of Biotechnology Department

To confirm hybridity another 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.

3.2 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.

Table 1. Name and sources of *Luffa acutanguala* (ridge gourd) genotypes

Sl. No	Name of the Genotypes	Sources
01	BARI Jhinga-1	Bangladesh Agriculture Research Institute, Gazipur.
02	RIG-19\2	Savar, Dhaka
03	RIG47\2	Savar, Dhaka
04	RIG 83\2	Savar, Dhaka
05	KRG 02	Savar, Dhaka
06	KRG 04	Savar, Dhaka
07	Purbachal	Savar, Dhaka

BARI = Bangladesh Agriculture Research Institute

Table 2. Name and sources of sponge gourd genotypes

Sl. No	Name of the Genotypes	Sources
1	Bogra local	Bogra District
2	Faridpur local	Faridpur District
3	Lalmonirhat local	Lalmonirhat District

Intercultural operation and crop production

3.3 Land preparation

The land, which was selected to conduct the experiment, was opened on 20 March, 2017 by rotary plough. After opening the land with a tractor it was ploughed and cross ploughed four times with a power tiller and laddering to break up the soil clods to level the land.

3.4 Layout and planting pattern

Raised bed was prepared with 15m in length and 3.5m in width and 0.5m trench between two beds. Five pits were prepared in each line. Pit to Pit distance was 3m and pit size was 45m X 45m X 30m. In total, 40 pits were prepared for transplantation of seven ridge gourd seedlings and three sponge gourd seedlings. The pits were left open for one week prior to transplanting. Two plants were transplanted in each pit.

Cowdung, Urea TSP, MoP, Boron fertilizers and furadan were used at recommended dose.

3.5 Sowing of seeds and transplanting of seedlings

Seeds were sown in polybags having compost mixed soil on 10 March 2017 for germination and seedling raising. Three seeds were sown in each polybag.

Before sowing seeds were soaked in water for 24 hours so that the seed germinated easily. The polybags were kept in shady place. Around 4 to 5 days required for germination after seed sowing. They were watered regularly during the seedling-raising period. When the seedlings (14 days old) attained 4 leaves and hard enough, they were transplanted in the main field on 29 March 2017.

3.6 Intercultural operation

Gap filling, weeding and crop management

Dead, injured and weak seedlings were replaced by new vigor seedlings from the same stock of the experiment. Mechanical weeding was done by Nirani whenever it was necessary to keep the plots free from weeds. Irrigation was given frequently at primary stage but less at later stage due to rainfall. Stormy weather may broke the tendering vine of the plants. For proper growth and development of the plants the vines were tied upward with the help of bamboo sticks. There was a plan to protect the plant from the attack of insects-pests specially fruit flies and fruit borer by spraying of pesticides. Pheromone trap and pesticides were used as required doses. Bagging was also done to protect the fruits. With the help of bamboo poles and iron rope stage was prepared for each line keeping 5 feet high from the ground level.

3.7 Crossing between ridge gourd and sponge gourd

Crossing among different genotypes and also selfing were done during May 01 to 30 July 2017. For crossing female flowers of ridge gourd were bagged in the morning and female flowers of sponge gourd were bagged at the afternoon one day before anthesis. The flowers were pollinated with pollen from the desired male plant during 5 p.m. to 7 p.m. and 6.00 a.m. to 8.00 a.m. Hand pollination was done by dusting pollen just after dehiscence of anther. The female flowers were immediately rebagged for 3-4 days (Plate 1 A-D). Records on fruit setting were noted and the fruits were harvested at maturity.

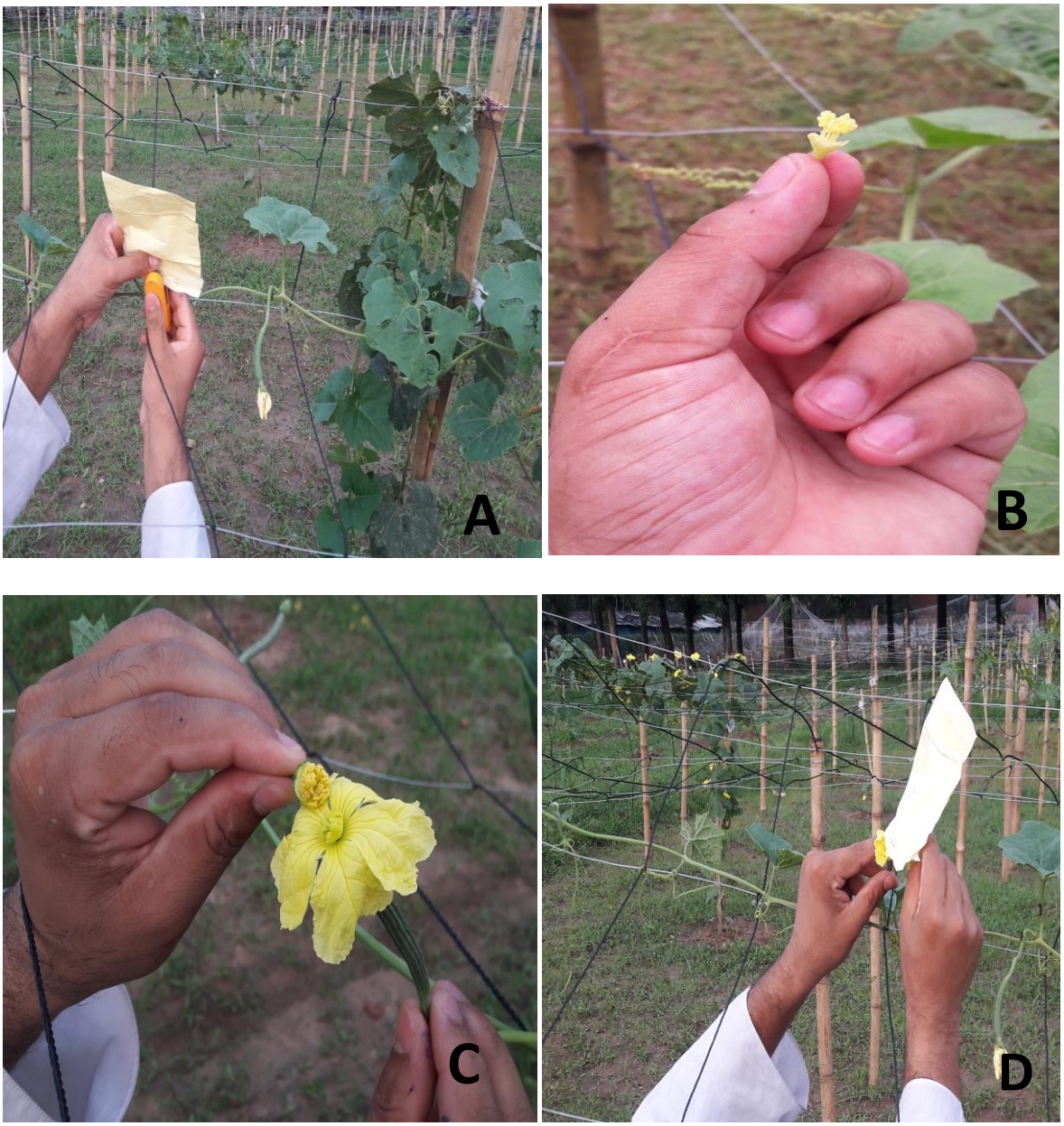


Plate 1: Crossing between ridge gourd and sponge gourd (Ridge gourd used as female)

A) Bagging of unopened female flower of ridge gourd B) Removing of petals from male flower of sponge gourd C) Controlled Pollination or crossing between ridge gourd and sponge gourd D) Re-bagging of pollinated flower of ridge gourd.

3.8 Harvesting

Harvesting was done at seven days interval from every plant of every plot for collecting data.

Collection of seed from mature fruit

Seed of the hybrids were collected and used for sowing in next season to observe the offspring performance. To evaluate performance same parameters were observed.

3.9 Collection of experimental data in respect of fruit morphology and yield performance

Days to first flowering

Number of days from sowing to first flowering (male and female) was recorded for every plant in ridge gourd and sponge gourd.

Number of male and female flower

Total number of male and female flowers was counted individually. It was done at two days interval after first flowering to ensure all flowers to be counted.

Male and female flower ratio

Ratio of male and female flower was counted by dividing the number of male flower by female flower.

Length and diameter of fruit (cm)

Length and diameter of ten randomly selected fruits per plant was measured after each harvest and then the average was taken.

Weight per fruit (g)

After each harvest, individual weight of randomly selected ten fruits per plant was recorded and then the average weight of fruit was calculated.

Total number of fruit per plant

Total number of fruit was counted and average fruit number was calculated.

Yield per plant (kg)

Yield per plant was recorded in kg.

Total number of crosses

Crossing between all possible combinations were done by controlled hand pollination between the genotypes of ridge gourd and sponge gourd. Data was recorded and calculated.

Number of fruits set

Number of fruits set per plant allowing artificial pollination were calculated. The success and failure of fruit setting were observed by counting the initial flower number and finally developed fruits.

Number of seeds per fruit

Five ripe fruits of each genotype were randomly selected and the number of seeds per fruit were counted. Seed number of fruit from cross was also counted.

For molecular assessment of hybridity in F_1 generation

3.10 Leaf sample collection

In order to molecular characterization fresh and young leaf samples were collected at 3-4 leaf stage of each genotype 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 ^{0}C freezer.

3.11 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.

3.11.1 Reagents Used

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. TBE buffer

Composition of 5 x TBE buffer (1 liter)

- 54 g of Tris base
- 27.5 g of Boric acid
- 4.65g of EDTA
- PH = 8.3

5. Isopropanol

6. 0.3 M Sodium Acetate

7. Absulate (100) ethanol

8. 70% Ethanol

9. RNAase

10. Ethidium Bromide solution.

3.11.2 Reagent Preparation for DNA Extraction (Stock Solution)

Extraction buffer (1000 ml)

- 100 ml 1M TrisHCl (pH 8.0) was taken in a measuring cylinder.
- Then 40 ml of 0.5M EDTA was added.
- 100 ml 5M NaCl was mixed with the mixture.
- Finally sterilized ddH_2O was added to make the volume up to 1000 ml.
- Then the mixture was mixed well and autoclaved.

1M Tris-HCl, pH 8.0 (250 ml)

- At first 30.28g Tris was taken in a volumetric flask (2500 ml)
- 100 ml ddH_2O was added.
- PH was adjusted to 8.0 by adding HCl.
- Then sterilized ddH_2O was added to make the volume up to 250 ml and the solution was autoclaved.

0.5M EDTA, pH = 8.0 (250 ml)

- At first 46.53g EDTA.2H_2O was added in a volumetric flask (250 ml)
- 100 ml ddH2O was added.
- Then 4 g NaOH was added.
- PH was adjusted to 8.0 with NaOH
- Then sterilized ddH_2O was added to make the volume up to 250 ml.
- The solution was autoclaved.

5M NaCl (250 ml)

- Firstly 73.05 g of NaCl was added in 250 ml ddH2O.
- It was then mixed well and autoclaved.

1% SDS (100 ml)

- 1 g of SDS was added in 100 ml ddH2O in a 250 ml beaker.
- As SDS is hazardous, so the mixture was mixed by a hot plate magnetic stirrer well. The solution was not autoclaved.

1X TE Buffer (100 ml)

- 1 ml Tris (pH 8.0) was taken in a volumetric flask (250 ml)
- Then 0.2 ml of 0.5M EDTA (pH =8.0) was added.
- Sterilized ddH_2O was added to make the volume up to 100 ml

Phenol: Chloroform: Isoamyl Alcohol= 25:24:1 (100 ml)

- At first 50 ml Phenol was taken in a volumetric flask (100 ml).
- Then 48 ml Chloroform was added.

- 2 ml Isoamyl Alcohol was also added and mixed well.
- The solution was stored at 4°C.

RNase A/H

- 10 mg of RNase was added to 1 ml of ddH_2O.
- Then it was dissolved completely with the help of necessary heat (at 50 °C in water bath for 30 min)

70% Ethanol (500 ml)

- 350 ml absolute ethanol was mixed with 150 ml dd.H2O

3.11.3 Sequential steps for DNA extraction

1. For Isolation of genomic DNA, vigorous, young, actively growing fresh leaf tissues were collected from 7 different ridge gourd and three different sponge gourd and their progeny of interspecific crosses varieties. Total DNA was isolated by using Phenol: Chloroform: Isoamyl alcohol method and ethanol precipitation method.
2. Initially, healthy youngest leaves were washed thoroughly by tap water followed by deionized water. Then sterilized by ethanol to remove wastes and any source of foreign DNA and leaves were then dried on tissue paper.
3. Approximately 200 mg of young leaves were cut into small pieces and then taken in morter. 600 µl of extraction buffer was added to it. The ground samples were taken into the 1.5 ml eppendorf tube.
4. Then it was vortexed for 20 seconds in a vortex mixture and then incubated at 65°C for 20 minutes in hot water bath.
5. Equal volume (600 µl) of Phenol: Chloroform: Isoamyl Alcohol (25: 24: 1) was added to the tube. Then it was vortexed for 20 seconds.
6. The solution was then centrifuged for 10 minutes at 13000 rpm. The supernatant was recovered using a pipette tip without disturbing the lower portion and transferred into a new eppendorf tube.
7. Approximately 400-450 µl was taken and then equal volume of Chloroform: Isoamyl Alcohol (24: 1) was added to it. The solution was vortexed for 10 seconds.
8. Again the solution was centrifuged at 13000 rpm for 10 minutes.

9. The supernatant was taken in a separate eppendorf tube and the lower layer was discarded.
10. Isopropanol was added about 0.6 volume of the liquid.
11. It was then gently tapped by finger for 20-30 seconds (The genomic DNA was visible as cotton like structure).
12. After tapping the sample was again centrifuged at 13000 rpm for 15 minutes. The liquid was discarded completely and re-precipitation of the DNA solution was done by adding 500µl of absolute (100%) cold ethanol plus 20µl 0.3 M Sodium acetate
13. It was shaken gently and tapping was done to separate pellet. The sample was centrifuged at 13000 rpm for 15 minutes. The liquid was removed completely by pouring and blotting the open tube end on fresh tissue paper.
14. The DNA pellet was then air dried for 2-3 hours. Then this pellet was dissolved in an appropriate volume (30 to 40µl) of TE buffer and treated with 3-4µl of RNAse for removing RNA. Then it was spinned for 4-5 seconds and incubated at 37°C for 20 munites.
15. Finally, the DNA samples were stored in freezer at -20°C.

3.12. Confirmation of extracted DNA

To confirm the extracted DNA sample 1% Agarose gel, working sample of each genomic DNA, 2x loading dye and deionized H_2O are needed.

DNA confirmation components with amount

Components	Amount (µI)
Working DNA sample	3.0
2x loading dye	3.0
Sterilized distilled water (ddH_20)	1.0
Total	7.0

Working solution of DNA samples preparation

DNA concentration was adjusted to 25 ng/µl for conducting PCR using the following formula: $V_1 \times S_1 = V_2 \times S_2$

Where,

V_1 = Initial volume of DNA solution (µl)

S_1 = Initial DNA concentration (ng/µl)

V_2 = Final volume of DNA solution (µl)

S_2 = Final DNA concentration (ng/µl)

3.12.1 1 % Agarose gel preparation

Reagents

- Agarose powder
- 5x TBE buffer (pH 8.3)
- Ethidium Bromide

Procedure

600 mg of agarose powder was taken in a 500 ml Erlenmeyer flask containing 60 ml electrophoresis buffer (Ix TBE buffer). 1x TBE buffer was prepared by adding 100 ml of 5x TBE buffer in 400 ml of de-ionized water. The flask was enclosed with aluminum foil paper to prevent excessive evaporation. The flask was heated in a microwave oven for about 2 minutes to generate uniform suspension until no agarose particle was seen to generate homogenous and crystal clear suspension. About 0.75µl ethidium bromide (DNA stain) was added and mixed well by gentle shaking to make the DNA visible under ultraviolet light box (Tran illuminator). The agarose solution was cooled to about 45-50^{0}C (flask was cool enough to hold comfortably with bare hand) and The molten gel was poured immediately on to a clean gel bed (l5cm × 10cm × 2 cm size). That was placed on a level bench and appropriate comb was inserted parallel to the plate's edge with the bottom of the teeth about 2 mm above the plate. After 25 minutes, gel was completely cooled at room temperature and solidified and the comb was removed gently. The gel was then ready for loading the DNA samples.

3.12.2. Preparation of DNA samples for electrophoresis

The samples were all in the same concentration in buffer. For each sample, 3 µl sample, 3 µl sterilized distilled H_20 and 2 µl of 2x loading dye (0.255 xylene ethanol, 0.255 bromophenol blue, 30% glycerol and 1mM EDTA) were taken in an eppendorf tube using 0.5-10 µl 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.

3.13 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 Gel Cam camera, DNA samples those showing better quality bands were taken for quantification and working solution preparation for next process.

3.14. Amplification of SSR markers by PCR

3.14.1. List of SSR primers

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.

Table 3. List of SSR primers, their sequences and GC content with Annealing temperature

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

3.14.2 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). Composition of 10.0 µl reaction mixture containing ready mix Taq DNA polymerase is given in Table 4.

Table 4. PCR reaction mixture composition for each genotype

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

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, water and mixed up well. The tubes were then sealed and placed in a thermo cycler and the thermo cycler was switched on immediately.

3.14.3. 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 95^0C for 4 min; denaturation with 95^0C for 40 sec,

annealing at 47^0C 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.

3.14.4. 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.

3.14.5 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 F1 hybrids.

3.15 Precautions

- To maintain a strategic distance from all types of contaminations and keep purity of DNA all dishes, micropipette tips, eppendorf tubes, glass pipettes, deionized water and buffer solutions were legitimately autoclaved. Metal supplies i.e., scissors, forceps were cleaned with absolute ethanol.

- Since Ethidium Bromide (Et-Br) is a serious mutagenic and carcinogenic in nature, hand gloves were utilized when taking of anything that has been presented to Et-Br.

- Always power pack was kept turn off and the leads were unplugged before opening the electrophoresis unit to avoid electrical hazard.

- Eye protector was used while working with trans-illuminator as it produces UV radiation of 254 nm range which can cause eye damage.

- The common safety measures were kept up when performing PCR responses.

All the disposable elements such as PCR tubes, tips, eppendorf tubes and reagents used during preparation of PCR reactions were autoclaved. Freezing condition was maintained when necessary. Hand gloves were worn at the time of amide treatment of PCR segments.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter comprises the presentation and discussion of the results of the experiment. Morphological observation of flower in both *Luffa acutangula* and *Luffa cylindrica* and fruit set in the crossing was recorded. In the SSR analysis significant genetic variation and polymorphisms for characterization of different ridge and sponge gourd cultivars were identified. The results of the experiment are presented in Table 5 to 9 and Plate 2 to 13 for ease of understanding.

4.1 Days to first flowering

Days to first male flowering ranged from 37 to 59 days in ridge gourd which was lowest in purbachal (37.5) genotype and highest was in RIG 83-2 (59) genotype. Days to first male flowering observed in case of sponge gourd that ranged 43 to 48 days but in case of hybrid Bogra × BARI Jhinga-1 days to first male flowering was observed 52 (Table 5).

Table 5. Days to first male and female flowering

Sl. No	Name of the Genotypes	Days to first male flowering	Days to first female Flowering
01	BARI Jhinga-1 (*L. acutangula*)	47.5	41
02	RIG-19\2 (*L. acutangula*)	49	50.5
03	RIG47\2 (*L. acutangula*)	58.5	61
04	RIG 83\2 (*L. acutangula*)	59	--
05	KRG 02 (*L. acutangula*)	41	40
06	KRG 04 (*L. acutangula*)	48.5	52
07	Purbachal (*L. acutangula*)	37.5	40
08	Bogra (*L. cylindrica*)	43.6	34.5
09	Faridpur (*L. cylindrica*)	48	48
10	Lalmonirhat (*L. cylindrica*)	47.5	47
11	Bogra × BARI Jhinga-1 (Hybrid)	52	53

Plate 2. Appearance of male flower in ridge gourd

Days to first female flowering ranged from 40 to 64 days in ridge gourd which was lowest in purbachal and KRG 02 (40) genotype and highest was in RIG 47/2 (61) genotype. Days to first female flowering observed in case of sponge gourd that ranged 34 to 48 which was highest in Faridpur genotype (48) and lowest in Bogra genotype (34). In case of hybrid of Bogra × BARI Jhinga-1 days to first female flowering was observed 53 (Table 5).

4.2 Number of male and female flower

Total number of male and female flowers was counted from ten randomly selected plants per plot. It was done at five days interval after first flowering to ensure all flowers to be counted.

Number of male flower varied significantly among the genotypes. In case of ridge gourd the highest number of male flower was observed in BARI Jhinga-1 which was 544.5 and lowest number of male flower was observed in RIG 83\2 (33.5). In case of sponge gourd the highest number of male flower was observed in Faridpur (262) and lowest number of male flower was observed in Lalmonirhat which was 17.5. Hybrid of Bogra × BARI Jhinga-1 gave 107 male flower (Table 6).

Table 6. Number of male and female flowers

Sl. No	Name of the Genotypes	Number of male flower	Number of female flower
01	BARI Jhinga-1 (*L. acutangula*)	544.5	48
02	RIG-19\2 (*L. acutangula*)	274	13.5
03	RIG47\2 (*L. acutangula*)	33.5	5
04	RIG 83\2 (*L. acutangula*)	33.5	-
05	KRG 02 (*L. acutangula*)	299.5	32.5
06	KRG 04 (*L. acutangula*)	212.5	25
07	Purbachal (*L. acutangula*)	255.5	25
08	Bogra (*L. cylindrica*)	127.5	24.5
09	Faridpur (*L. cylindrica*)	262	19.5
10	Lalmonirhat (*L. cylindrica*)	17.5	3.5
11	Bogra × BARI Jhinga-1 (Hybrid)	107	25.5

Number of female flower also varied significantly among the genotypes. In case of ridge gourd the highest number of female flower was observed in BARI Jhinga-1 which was 48 and lowest number of male flower was observed in RIG 83\2 (0). So RIG 83\2 was a male line. In case of sponge gourd the highest number of female flower was observed in Bogra (24.5) and lowest number of female flower was observed in Lalmonirhat which was 3.5. Hybrid of Bogra × BARI Jhinga-1 produced 25.5 female flower (Table 6).

4.3 Male and female flowers ratio

Male and female flower ratio varied significantly among the genotypes. In case of ridge gourd the highest Male and Female flower ratio was 20.3:1 in RIG-19\2 and the lowest was observed in RIG47\2 (6.7:1). In case of sponge gourd the highest Male and Female flower ratio was 13.5:1 in Faridpur and the lowest was observed in Lalmonirhat (5:1). Male and female flower ratio in hybrid of Bogra × BARI Jhinga-1 was 4.2:1. (Table 7).

Table 7. Male and female flower ratio

Sl. No	Name of the Genotypes	Male and female flower ratio
01	BARI Jhinga-1 (*L. acutangula*)	11.4:1
02	RIG-19\2 (*L. acutangula*)	20.3:1
03	RIG47\2 (*L. acutangula*)	6.7:1
04	RIG 83\2 (*L. acutangula*)	Only male
05	KRG 02 (*L. acutangula*)	9.2:1
06	KRG 04 (*L. acutangula*)	8.5:1
07	Purbachal (*L. acutangula*)	10.2:1
08	Bogra (*L. cylindrica*)	5.2:1
09	Faridpur (*L. cylindrica*)	13.5:1
10	Lalmonirhat (*L. cylindrica*)	5:1
11	Bogra × BARI Jhinga-1 (Hybrid)	4.2:1

A) Male flower of ridge gourd **B) Male flower of sponge**

C) Female flower of ridge gourd **D) Female flower of sponge gourd**

Plate 3. Male and female flowers of ridge gourd and sponge gourd

4.4 Total number of fruits per plant

Total number of fruits varied significantly among the genotypes. In case of ridge gourd the highest number of fruit was observed in BARI Jhinga-1 which was 42.5 and lowest number of fruit was observed in RIG 83\2 (0). In case of sponge gourd the highest number of fruit was observed in Faridpur (16.5) and lowest number of fruit was observed in Lalmonirhat which was 0. Total 22 fruits was harvested from hybrid of Bogra × BARI Jhinga-1 gave (Table 8).

Table 8. Total number of fruits per plant

Sl. No	Name of the Genotypes	Total number of fruits per plant
01	BARI Jhinga-1 (*L. acutangula*)	42.5
02	RIG-19\2 (*L. acutangula*)	12
03	RIG47\2 (*L. acutangula*)	4
04	RIG 83\2 (*L. acutangula*)	0
05	KRG 02 (*L. acutangula*)	26.5
06	KRG 04 (*L. acutangula*)	22
07	Purbachal (*L. acutangula*)	20.5
08	Bogra (*L. cylindrica*)	19
09	Faridpur (*L. cylindrica*)	16.5
10	Lalmonirhat (*L. cylindrica*)	0
11	Bogra × BARI Jhinga-1 (Hybrid)	22

4.5 Length and diameter of fruit

Length of ten randomly selected fruits per plant was measured after each harvest and then the average was taken. Diameter of the ten randomly selected fruits harvested was measured and the average was calculated in cm. The maximum length observed in ridge gourd was 28.75 cm in BARI Jhinga-1 and the minimum in Purbachal (19.85 cm). In case of sponge gourd the highest length observed in Faridpur (27.15 cm) and the lowest was in Bogra (25.30 cm). The highest diameter observed in ridge gourd was 4.85cm in BARI Jhinga-1and

RIG47\2 and the lowest was in RIG-19\2 (3.05cm). In case of sponge gourd the highest diameter observed in Bogra (5.1cm) and the lowest was in Faridpur (3.95cm).The length and diameter of the hybrid of Bogra×BARI Jhinga-1 was 25.15cm and 4.3cm respectively (Table 9).

Table 9. Length and diameter of fruit

Sl. No	Name of the genotypes	Length of fruit (cm)	Diameter of fruit (cm)
01	BARI Jhinga-1 (*L. acutangula*)	28.75	4.85
02	RIG-19\2 (*L. acutangula*)	21.4	3.05
03	RIG47\2 (*L. acutangula*)	26.85	4.85
04	RIG 83\2 (*L. acutangula*)	--	--
05	KRG 02 (*L. acutangula*)	25.3	3.4
06	KRG 04 (*L. acutangula*)	27.05	4.1
07	Purbachal (*L. acutangula*)	19.85	3.55
08	Bogra (*L. cylindrica*)	25.30	5.1
09	Faridpur (*L. cylindrica*)	27.15	3.95
10	Lalmonirhat (*L. cylindrica*)	--	--
11	Bogra×BARI Jhinga-1 (Hybrid)	25.15	4.3

4.6 Weight per fruit (g)

After each harvest, the weight of randomly selected ten fruits per plant was recorded and then the average weight per fruit was calculated. The maximum weight was 226.65 gm observed in BARI Jhinga-1 and the minimum was in RIG-19\2 (77.1 gm). In case of sponge gourd the maximum weight observed in Bogra (201.9gm) and the minimum was in Faridpur (201.5 gm). The weight of the hybrid of Bogra×BARI Jhinga-1 was 226gm (Table 10).

Table 10. Weight per fruit (gm)

Sl. No	Name of the genotypes	Weight per fruit (gm)
01	BARI Jhinga-1 (*L. acutangula*)	226.65
02	RIG-19\2 (*L. acutangula*)	77.1
03	RIG47\2 (*L. acutangula*)	207
04	RIG 83\2 (*L. acutangula*)	--
05	KRG 02 (*L. acutangula*)	158
06	KRG 04 (*L. acutangula*)	175
07	Purbachal (*L. acutangula*)	97
08	Bogra (*L. cylindrica*)	201.9
09	Faridpur (*L. cylindrica*)	201.5
10	Lalmonirhat (*L. cylindrica*)	--
11	Bogra×BARI Jhinga-1 (Hybrid)	226

Plate 04. Hybrid fruit (Bogra×BARI Jhinga-1) having 217gm weight

4.7 Yield per plant (kg)

In case of yield per plant the maximum yield was observed in BARI Jhinga-1 which was 9.3kg/plant and the minimum was in RIG47\2 (0.83 kg/plant). In case of sponge gourd the maximum yield observed in Bogra (3.84 kg/plant) and the

minimum was in Faridpur (3.33 kg/plant). The yield of Bogra×BARI Jhinga-1 hybrid was 4.97 kg/plant (Table 11).

Table 11. Yield per plant (kg)

Sl. No	Name of the genotypes	Yield per plant (kg)
01	BARI Jhinga-1 (*L. acutangula*)	9.63
02	RIG-19\2 (*L. acutangula*)	0.93
03	RIG47\2 (*L. acutangula*)	0.83
04	RIG 83\2 (*L. acutangula*)	--
05	KRG 02 (*L. acutangula*)	4.19
06	KRG 04 (*L. acutangula*)	3.85
07	Purbachal (*L. acutangula*)	1.87
08	Bogra (*L. cylindrica*)	3.84
09	Faridpur (*L. cylindrica*)	3.33
10	Lalmonirhat (*L. cylindrica*)	--
11	Bogra×BARI Jhinga-1 (Hybrid)	4.97

4.8 Physical appearance of fruit

It observed that the hybrid fruit of Bogra×BARI Jhinga-1 was intermediate between Bogra and BARI Jhinga-1. Ridges in BARI Jhinga-1 was more prominent and in Bogra ridges was smooth. The ridges of the hybrid fruits was neither prominent nor smooth (Plate 5).

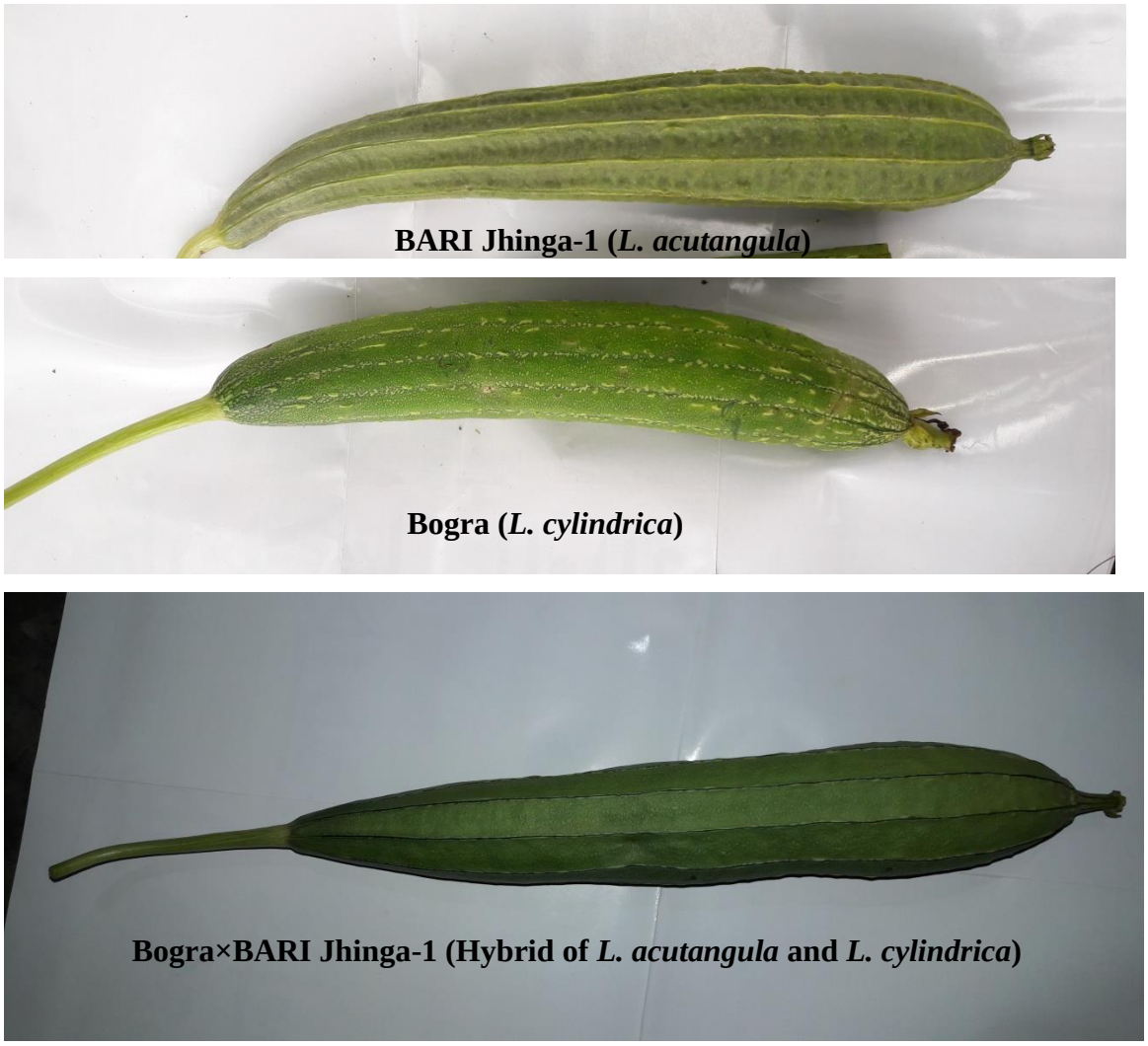
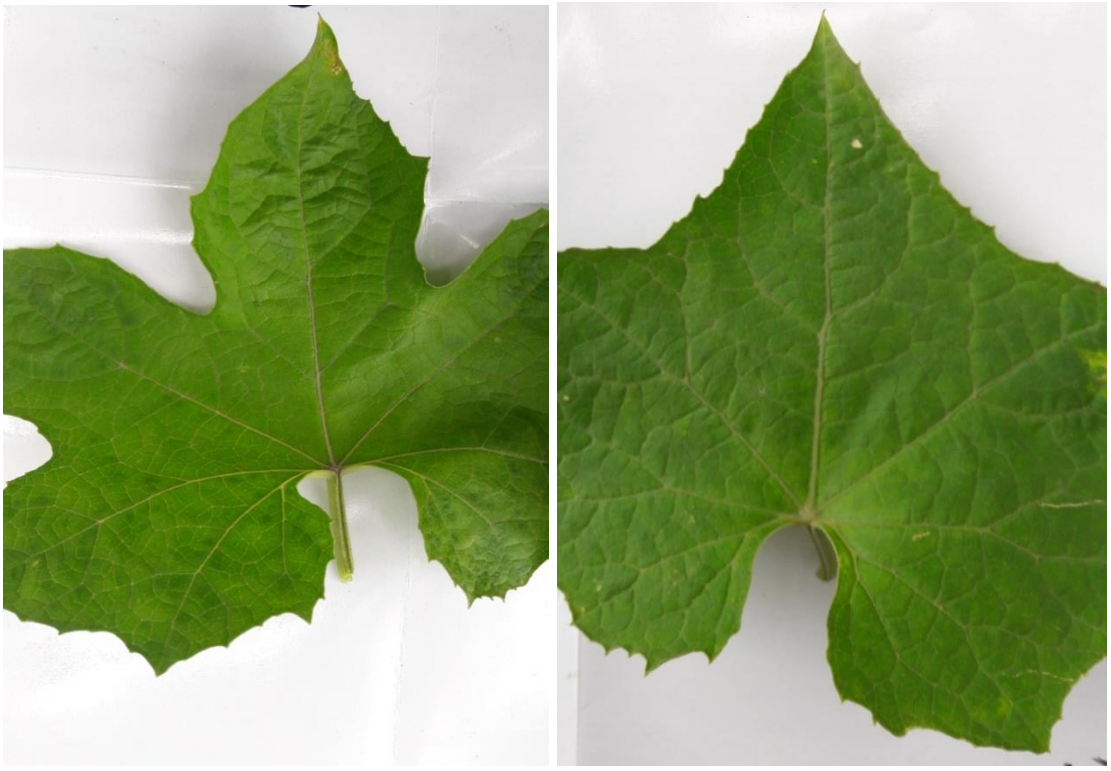


Plate 5. Physical appearance of hybrid fruit (Bogra× BARI Jhinga-1) and its corresponding parents

Leaf observation in ridge gourd and sponge gourd and their interspecific progeny

The size of the leaf approx. in parental genotype of BARI Jhinga-1 was 13.5cm×8.9cm and in Bogra was 17.6cm×11.4cm. The leaf size in hybrid of Bogra×BARI Jhinga-1 was 13.1cm× 10.5cm. The other characters (color, smoothness, leaf blade etc.) of the leaf of the hybrid (Bogra×BARI Jhinga-1) was intermediate between Bogra and BARI Jhinga-1 (Plate 6).



Leaf of parents A) Leaf of BARI Jhinga-1 B) Leaf of Bogra genotype

Leaf of hybrid of Ridge gourd×Sponge gourd (Bogra×BARI Jhinga-1)

Plate 6. Leaf of hybrid (Bogra× BARI Jhinga-1) and its corresponding parents

4.9 Number of Seed:

Seeds were extracted and counted after harvesting of fruit. All of the fruits of compatible crosses were harvested and seeds were extracted. In case of ridge gourd the highest number of seed was observed in RIG 19-2 which was 233.5 and lowest number of seed was observed in RIG 83\2 (132). In case of sponge gourd the highest number of seed was observed in Faridpur (209.5) and lowest number of seed was observed in Bogra which was 140. Hybrid of Bogra × BARI Jhinga-1 gave 80.5 seeds. The Bogra×RIG 19-2 produce highest number of seeds (230.5) and Bogra×RIG 83-2 produce lowest number of seeds (74) among the crosses (Table 12).

Table 12. Number of Seeds per fruit

Sl. No	Name of the Genotypes	Number of seeds per fruit
01	BARI Jhinga-1 (used as male parent)	173
02	RIG 19-2 (used as male parent)	233.5
03	RIG 47-2 (used as male parent)	154
04	RIG 83-2 (used as male parent)	--
05	KRG 02 (used as male parent)	199.5
06	KRG 04 (used as male parent)	197
07	Purbachal (used as male parent)	204.5
08	Bogra (used as female parent)	140
09	Faridpur (used as female parent)	209.5
10	Lalmonirhat (used as female parent)	--
11	Bogra× BARI Jhinga-1(hybrid)	80.5
12	Bogra×RIG 19-2 (hybrid)	230.5
13	Bogra×RIG 47-2 (hybrid)	65.5
14	Bogra×RIG 83-2 (hybrid)	74
15	Bogra×KRG 02 (hybrid)	166
16	Bogra×Purbachal (hybrid)	197
17	Faridpur×RIG 19-2 (hybrid)	86
18	Faridpur×KRG 02 (hybrid)	111
19	KRG 02×Bogra (hybrid)	170

Plate 7. Seeds of hybrid and its corresponding parents

4.10 Cross compatibility percentage

Total number of crosses was counted and the number of successful crosses was also counted. The maximum result was found in Bogra× BARI Jhinga-1 (75%) and the minimum result was found in Faridpur×BARI Jhinga-1, Lalmonirhat×BARI Jhinga-1, Lalmonirhat×RIG 47/2 (0%) (Table15). Causes of incompatibility are unknown. Scientists guise some causes those may be inability of the germination of pollen, pollen tube fails to enter inside the stigma, no fertilization due to degeneration of egg cells, embryo degenerate at very early stage etc.

Table 13. Success rate of interspecific crosses between *Luffa acutangula* and *Luffa cylindrica*

Sl. No.	Male	Female	Total number of crosses	No. of successful crosses	Success rate in percent
1	BARI Jhinga	Bogra	8	6	75
2	RIG-19\2	Bogra	2	1	50
3	RIG47\2	Bogra	6	4	66
4	RIG 83\2	Bogra	2	1	50
5	KRG 02	Bogra	3	1	33
6	Purbachal	Bogra	2	1	50
7	Bogra	Purbachal	1	0	0
8	BARI Jhinga-1	Faridpur	3	0	0
9	RIG-19\2	Faridpur	3	2	66
10	RIG47\2	Faridpur	2	1	50
11	KRG 02	Faridpur	2	1	50
12	BARI Jhinga-1	Lalmonirhat	3	0	0
13	RIG47\2	Lalmonirhat	2	0	0
14	Bogra	KRG 02	2	1	50

Molecular assessment for hybridity test

4.11. 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 (Plate 8) 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.

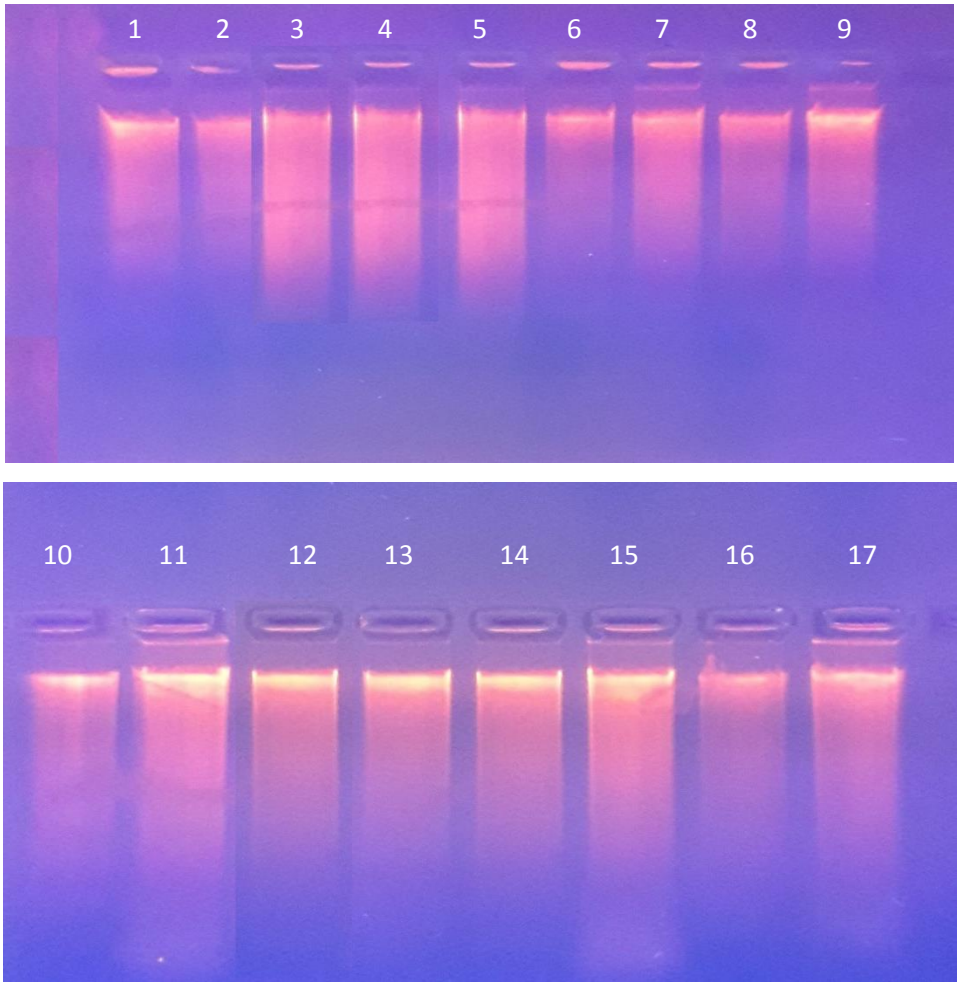


Plate 8: Genomic DNA from different genotypes of ridge gourd and sponge gourd and their hybrid

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 Lane18= KRG 04

4.12 Principle of 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.

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.

4.12 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 14 to 18.

Table 14. 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	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

Table 15. 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

Table 16: 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 17. 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

Table 18. 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 reveals that, four primers *viz* BoG 21, BoG 76, BoG 117 and BoG 127 showed DNA amplification in three hybrids and their corresponding parents.

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 (Plate 9).

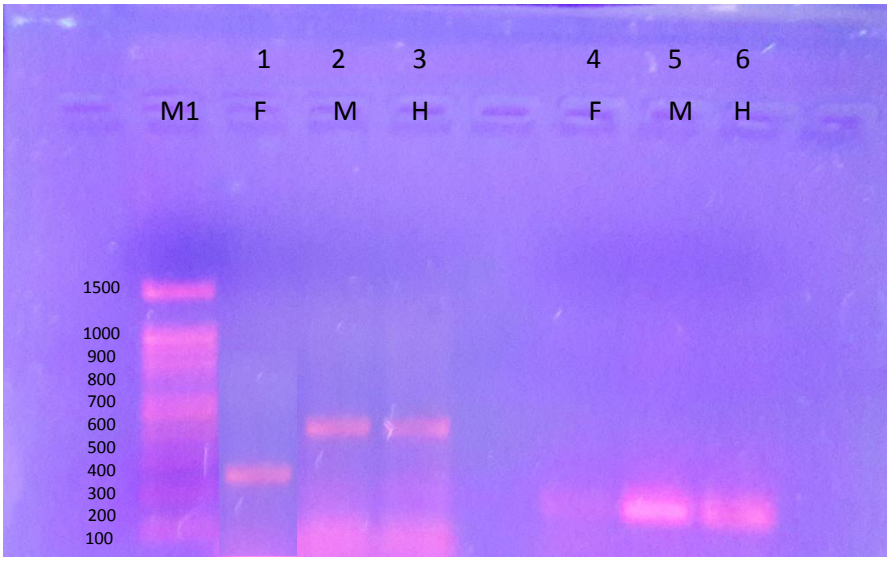


Plate 9: The SSR primer BoG 21 was used for hybrid identification

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)

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 (Plate 10).

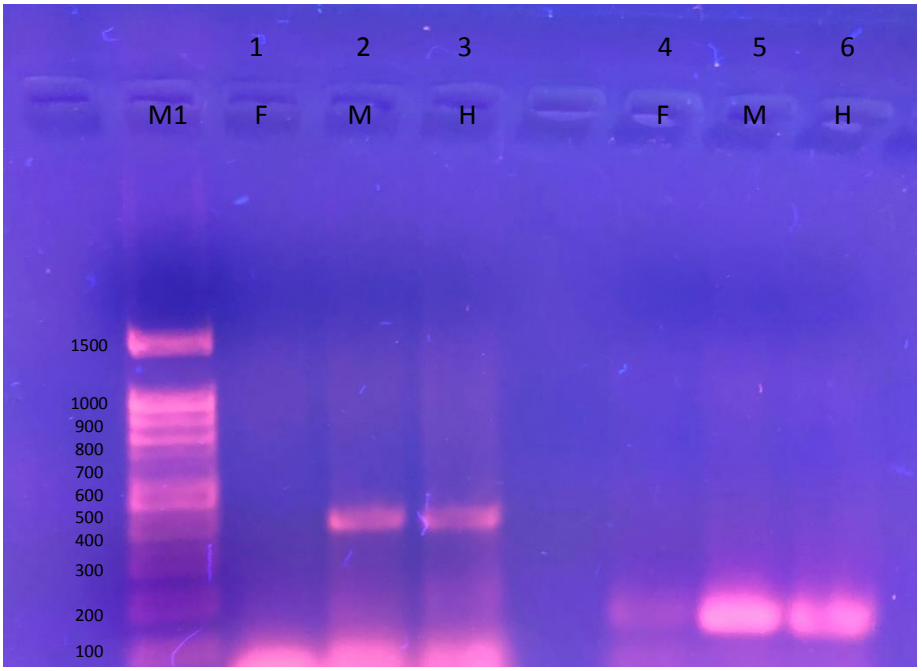


Plate 10: PCR amplification with SSR primer BoG 76

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)

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.

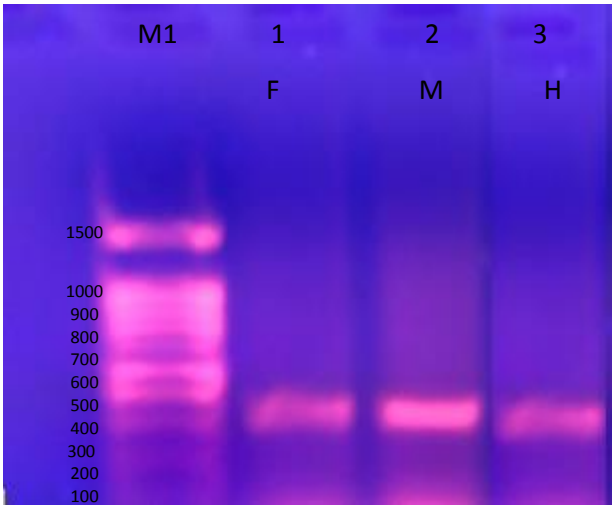


Plate 11: PCR with SSR primer BoG 117 in case of hybrid Faridpur×KRG 02

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

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.

It is interesting to note that, the primer BoG 21 produced polymorphic DNA fragment among the parents of the hybrid Bogra×RIG47-2 (Plate 9) 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 (Plate 10). 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.

CHAPTER V

SUMMARY AND CONCLUSION

Ridge gourd (*Luffa acutangula*) is one of the most important summer cucurbitaceous vegetable in Bangladesh. Ridge gourd is beneficial for minimizing constipation, lowering blood sugar, hypoglycemia, enhancing weight loss, boosting the immune system and detoxing etc.

Being comparatively less developed and poorly studied, little information is available on the cross compatibility between ridge gourd and sponge gourd. Characterization of these genotypes and identification of F_1 hybrids with molecular technique is a new approach of hybrid purity. This is why, there is a need to conduct studies to evaluate the compatibility and 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. The present experiment was conducted at the farm of Sher-e-Bangla Agricultural University and Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207.

Interspecific hybridization allows a transfer of genes of interest between different species. Seven ridge gourd and three sponge gourd varieties are used as parents and interspecific crosses. Most of the crosses were compatible viz. Bogra×BARI Jhinga-1, Bogra×RIG 47-2, Faridpur×RIG 19-2 etc. Few crosses were incompatible such as Lalmonirhat×BARI, Lalmonirhat×RIG 19-2 etc. Morphology of offspring of the compatible crosses are intermediate between ridge gourd and sponge gourd. The interspecific hybrids produced fruits with less number of seeds with less fiber density than that of the *L. cylindrica* entries.

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.

This study shows that SSR markers can be used to successfully detect hybrids in ridge gourd and sponge gourd. Screening SSR markers for polymorphism showed that different genotypes can be represented by different alleles which are helpful in identifying true hybrids among progenies. The use of SSR markers provided an early detection method to select and screen out plants even at an early stage of development.

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. 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.

RECOMMENDATION

The present work was the preliminary study to assess cross compatibility of ridge gourd and sponge gourd genotypes. It had some limitations in term of limited number of individuals and varieties, microscopic study of behavior of pollen, stamen and pistils as well as number of primers used. 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:

1. Large number of varieties and higher number of crosses should be done with adequate replications to increase accuracy.

2. Study should be conducted about the response and behavior of stamen and pistils and causes of cross incompatibility.
3. Details survey work should be conducted using more molecular markers for obtaining polymorphism among the parents.
4. Other molecular markers such as ISSR, AFLP, etc. should be used for further confirmation.

Cross compatibility study has a great value in case of gene transfer and hybrid variety development. Molecular technique for hybridity test is safer, more reliable and less time consuming in compare to field level hybridity. The developed protocol can be used for hybridity test in any crop. The commercial aspects of new innovation may be viable for all the hybrid seed development institute in Bangladesh.

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