DNA PROFILING AND MOLECULAR DIVERSITY ANALYSIS OF BRINJAL (*Solanum melongena* L.) CULTIVARS USING RAPD MARKERS

SHARMIN SULTANA

DEPARTMENT OF BIOTECHNOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
SHER-E-BANGLA NAGAR, DHAKA-1207, BANGLADESH

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BY

SHARMIN SULTANA
Registration No. 08-02810

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Approved by

…….	…….
Mohammad Nazrul Islam	**Professor Dr. Md. Ekramul Hoque**
Assistant Professor	**Co-supervisor**
Supervisor	

.....
Homayra Huq
Associate Professor
Chairman
Examination Committee

DEPARTMENT OF BIOTECHNOLOGY
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

CERTIFICATE

This is to certify that thesis entitled, **"DNA PROFILING AND MOLECULAR DIVERSITY ANALYSIS OF BRINJAL** (*Solanum melongena* **L.) CULTIVARS USING RAPD MARKERS."** **s***ubmitted to the Faculty of* **AGRICULTURE,** *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 bona fide research work carried out by* **SHARMIN SULTANA,** *Registration No.* **08-02810** *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 of during the course of this investigation has duly been acknowledged.

Date: December, 2014
Place: Dhaka, Bangladesh

(Mohammad Nazrul Islam)
Assistant Professor
Department of Biotechnology
Sher-e-Bangla Agricultural University

DEDICATED TO
MY
BELOVED PARENTS

ABBREVIATIONS AND ACRONYMS

Full word	Abbreviation	Full word	Abbreviation
2,4-Dichlorophenoxy acetic acid	2,4-D	Percent	%
Amplified Fragment Length Polymorphism	AFLP	Polymerase chain reaction	PCR
And others (at elli)	*et al.*	Quantitative trait loci	QTL
Angstrom	Å	Random Amplified Polymorphic DNA	RAPD
As for example	e.g.	Restriction Fragment length Polymorphism	RFLP
Bangladesh Agricultural Research Institute	BARI	Ribonucleic Acid	RNA
Bangladesh Bureau of Statistics	BBS	Rotation per minute	rpm
Base pair	bp	Sequence Tagged Site	STS
Centi Morgan	cM	Similarity Index	SI
Cetyl Trimethyl Ammonium Bromide	CTAB	Simple Sequence Repeat	SSR
Continued	Cont'd	Sodium chloride	NaCl
Degree celsius	°C	Sodium Dodecyl Sulphate	SDS
Deoxyribonucleic acid	DNA	Species (plural)	spp.
Distilled deionized water	ddH2O	Simple Sequence Repeat	SSR
Ethylene Diamine Tetra Acetic Acid	EDTA	That is	i.e.
Etcetera	etc.	*Thermophilus aquaticus*	Taq
Ethidium Bromide	Et-Br	Tris Boric Acid EDTA	TBE
Food and Agriculture Organization	FAO	Tris-EDTA	TE
Genetic Distance	GD	Un-weighted Pair Group Method of Arithmetic Mean	UPGMA
Gram per Liter	g/L	Ultra Violet	UV
Hectare	Ha	Volt	V
Micro liter	µl		
Micro mole	µM		
Mili liter	mL		
Namely	viz.		
Negative logarithm of hydrogen ion concentration (-log[H+])	pH		

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Dated: December, 2014. ***The Author***

SAU, Dhaka

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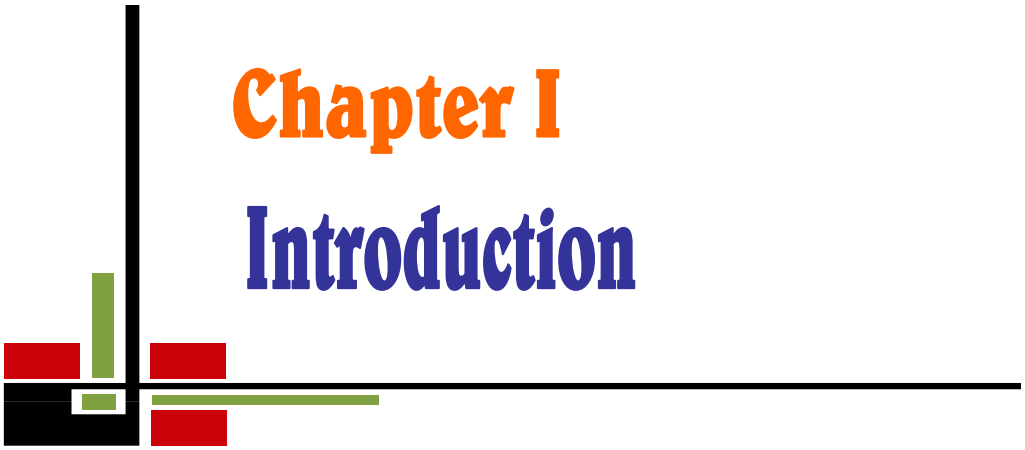
DNA PROFILING AND MOLECULAR DIVERSITY ANALYSIS OF BRINJAL (*Solanum melongena* L.) CULTIVARS USING RAPD MARKERS

ABSTRACT

An experiment was conducted on Brinjal (*Solanum melongena* L.) in Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka-1207. The genetic variation of 11 brinjal varieties (BARI begun 1, BARI begun 4, BARI begun 5, BARI begun 6, BARI begun 7, BARI begun 8, BARI begun 9, BARI begun 10, BARI hybrid begun 1, BARI hybrid begun 3 and BARI hybrid begun 4) were studied based on PCR technology using five RAPD primers. Sum total of 44 distinct DNA amplified band were observed with an average of 5.6 polymorphic bands per primer. The overall gene diversity was detected 0.2186 and level of polymorphism was detected 63.64%. The pair wise inter-variety similarity indices ranged from 67.03% to 97.61%. The UPGMA Dendrogram segregated 11 genotypes of brinjal into two main clusters. The first major cluster had only one genotype (BARI begun 6) and the second major cluster had rest of ten genotypes. BARI begun 6 vs. BARI begun 1 showed highest Nei's genetic distance (0.5261) as they are released from different parental origin. On the other hand BARI began 9 vs. BARI begun 7 varietal pair showed lowest genetic distance (0.0465) as they are released from same parental origin. The experiment reveals significant level of variations among 11 brinjal genotypes by RAPD markers. The results of this study can be used as a baseline for the future diversity assessment and genetic analysis of brinjal varieties of Bangladesh.



Chapter I

Introduction

CHAPTER I

INTRDUCTION

Brinjal (*Solanum melongena* L.) is an economically important Solanaceous vegetable, widely consumed in Asia, Europe, Africa and America (Ali *et al.*, 2011, Kumar *et al.*, 2008; Collonnier *et al.*, 2003). It is locally known as 'Begon' and its early European name is 'eggplant' and also known as guinea squash, garden egg and aubergine (Khan *et al.*, 2013). It is normally a self-pollinated, diploid (2n=2x=24) annual crop that belongs to the family Solanaceae (Thompson, 1951) a large plant family with more than 3,000 species and genus *Solanum*, a close relative of potato, tomato and pepper (Spooner and Knapp, 2013; Barrell *et al*., 2013).

Brinjal is one of the widely used vegetable crops and is popular in many countries *viz*. Central, South and South East Asia, some parts of Africa and Central America (Harish *et al*., 2011). It is an ancient crop. The cultivated brinjal is undoubtedly of Indian origin and has been in cultivation for a long time (Thompson and Kelly, 1957). Hundreds of cultivars with many wild types are available in this area (Sing *et al*., 2001). The medicinal and economic value of eggplant is found in Sanskrit literature (Kalloo, 1993; Hinata, 1986; Khan, 1979). It is originated in India, as Subcontinent people are used to grow brinjal since last 4000 years (Dunlop, 2006). Indo-Burma, China and Japan are the secondary centers of eggplant origin (Gleddie *et al.*, 1986). Arabs introduced eggplant to the west during the 15th century (Hinata, 1986). Eggplant germplasm resources and collections have been well documented, evaluated and conserved throughout the world (Sarathbabu *et al.*, 1999). Currently, brinjal is extensively grown in Bangladesh, India, Pakistan, U.A.E, Sri Lanka, Egypt and other warm countries of the world. It is also grown in Nepal, Japan, France, Italy, USA, the Mediterranean and Balkan area (Bose and Som, 1986).

In Bangladesh, brinjal is the second most important vegetable crop next to potato in respect of acreage and production (BBS, 2012). The total area of eggplant cultivation is 31565.48 ha while 13759.31 ha in Rabi season and 17806.17 ha in Kharif season with total annual production of 246000 tons and average yield is 15.81 t/ha (BBS, 2012). Although the crop is grown throughout the country, it is intensively and commercially grown in Jessore, Rajshahi, Narsinghdi, Dhaka, Comilla and Bogra districts (Azad *et al*., 2012). Brinjal occupied about 25.4% of total vegetables cultivation area in Bangladesh. At present, eggplant is ranked as third most important crop from Solanaceae family after potato and tomato in the world with an annual worldwide production of more than $41x10^6$ ton (FAO, 2010). More than 1,600,000 hectares (4,000,000 acres) are devoted to the cultivation of eggplant in the world.

Brinjal is nutritious vegetable and has got multifarious use as a dish item (Rashid, 1993). It is a good source of minerals and vitamins (particularly iron) making its total nutritional value comparable with tomato (*Solanum lycopersicum* L.) in terms of its total nutritive value (Singh and Kumar, 2007; Kalloo, 1993). It has higher calorie, iron, phosphorus and riboflavin contents than tomato (Shaha, 1989). One cup of raw eggplant contains 20 calories, 0.8 grams of protein, 4.82 grams of carbohydrate, 0.15 grams of fat and 2.5 grams of dietary fiber. The fruit and other parts of the plant are used in traditional medicine. (Kashyap *et al*., 2003). For example, tissue extracts have been used for the treatment of asthma, bronchitis, cholera and dysuria; fruits and leaves are beneficial in lowering blood cholesterol. It has potential health effects against cancer, aging, inflammation, and neurological diseases. Eggplant has been a staple vegetable in our diet since ancient times. It is an inexpensive but major food component of the human diet in the developing world, most particularly in India and China (Doganlar *et al.*, 2002). It has potentiality as raw material in pickle making and in dehydration industries (Singh *et al.*, 1963). Fried brinjal has some medicinal value to cure liver problem (Chauhan,

1981). Brinjal is a familiar vegetable crop for its easier cooking quality, better taste and lower market price.

The yield potential of eggplant in Bangladesh is very low compared to other countries due to the incidence of insect pests and diseases, which greatly hampered the production of eggplant (Das *et al*., 2000, Rashid, 2000; Khan *et al*., 1998). Eggplant is susceptible to several pests (fruit and shoot borer; *Leucinodes* sp.) and diseases (*Fusarium*, *Verticillium* and Bacterial wilt), nematodes (*Meloidogyne* sp.) as well as abiotic stress conditions (Kashyap *et al*., 2003; Sihachakr *et al*., 1994). In contrast, the majority of wild species are resistant to nearly all known pests and pathogens of eggplant and thereby are a source of desirable traits for crop improvement. Plant breeders have addressed these constraints by identifying resistant/tolerant germplasm, determining the genetics involved and the genetic map positions of the resistant genes. Comparative genomics and synteny analysis with closely related brinjals promises to further advance the knowledge of the brinjal genome and provide the breeders with additional genes and selectable markers for use in marker-assisted selection.

Molecular markers are reliable tools to characterize the DNA profile of plant genotypes to study the genetic diversity. In Bangladesh, genetic data on brinjal is not rich enough. So genetic status of this important crop is needed to be established and documented by using DNA markers which may provide valuable information for further breeding programme.

Molecular markers can provide information that can help to define the distinctiveness of species and their ranking according to the number of close relatives and their phylogenetic position. For these reasons, molecular markers are rapidly being adopted by crop improvement research globally as an effective and appropriate tool for basic and applied studies addressing biological components in agricultural production systems (Jones *et al*., 1997).

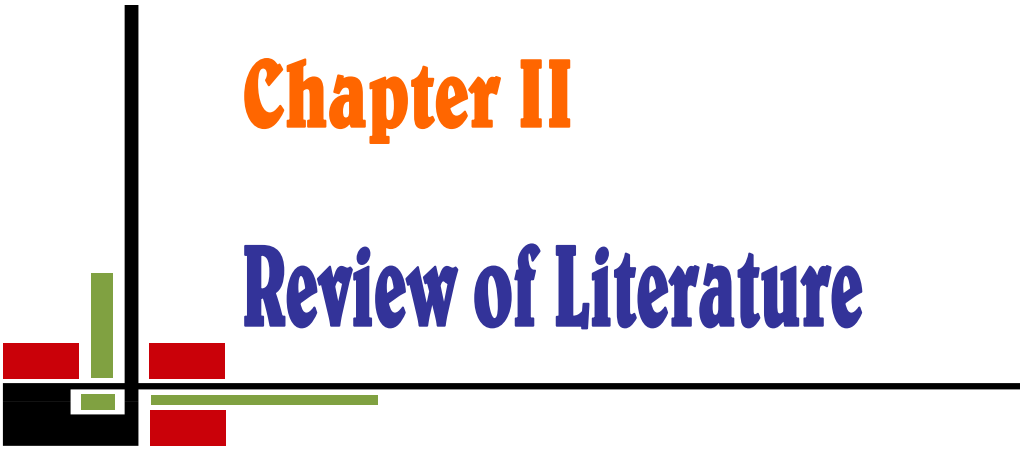
Among the different types of molecular markers available, randomly amplified polymorphic DNA (RAPD) markers are useful for the assessment of genetic diversity because of their simplicity and relatively low cost compared to other molecular markers (Williams *et al*., 1990; Rafalski and Tingey, 1993). The RAPD assay has advantages of being readily employed, requiring very small amounts of genomic DNA and eliminating the need for blotting and radioactive detection (Cipriani *et al*., 1996). Molecular characterization by RAPD markers is easy and rapid. RAPD markers have been widely used for the identification of genetic relationship among cultivars (Afzal *et al*., 2004; Tosti and Negri, 2002).

In spite of some weakness, the relative case and speed, the high degree of polymorphism and the virtually inexhaustible pool of possible genetic markers make the technique advantageous over the molecular techniques (Fristsch and Rieseberg, 1996; Clark and Lanigan, 1993).

The intension of this investigation was to assess genetic diversity and relatedness of brinjal cultivars by PCR based RAPD technique, as it is important particularly for variety selection for breeding purpose such as, hybridization, evaluation and conservation of their diverse gene pool.

Keeping these considerations in view, the present investigation was formulated with the following objectives:

1. To study genetic variation among the different released brinjal cultivars through RAPD markers.
2. Molecular characterization and varietal identification by DNA profiling.
3. To reveal phylogenetic relatedness and genetic distance among the brinjal genotypes.



Chapter II

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

Brinjal (*Solanum melongena* L.) is a widely cultivated and economically important vegetable crop of Bangladesh as well as all over the world. Great taste, high level of nutrition and considerable extent of medicinal value makes it worldwide popular. Wide morphological and genetic diversity exists in eggplant. Several researchers throughout the world have performed research activities on brinjal genetic variability and relationship, phylogenetic study and characterization through molecular markers like Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR) etc. Some of the research works also done in Bangladesh, but the genetic data on brinjal is not rich enough. For further breeding programme to improve the crop, more study should be carried out. The most relevant literatures about the present study have been reviewed and some of the most relevant literatures are cited below.

2.1 The concept of RAPD markers

Random amplified polymorphic DNA (RAPD) is a PCR-based marker system. RAPD markers are DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence. RAPD is a widely used approach for characterization of DNA from plants and other organisms. Short oligo-nucleotide primers of arbitrary (random) sequence are used in PCR reaction to generate amplified products. RAPD-PCR primers are not designed to amplify a specific target sequence, 'the amplified loci are anonymous and presumably scattered throughout the genome (Williams *et al.*, 1990; Tinker *et al*., 1993).

The RAPD-PCR technique has provided a relatively simple and inexpensive method for examining variation in the total genome (Hadrys *et al.*, 1992). RAPD analysis is advantageous over isozyme electrophoresis because it

generates much greater numbers of loci required for genetic analysis (Kimbeling *et al.,* 1996). RAPD markers can be used as supposedly unbiased; and neutral markers for genetic mapping applications (Michelmore, *et al.*, 1991), in population genetics (Haig *et al*., 1994), taxonomy (Chapco *et al.*, 1992) as well as for genetic diagnostics.

DNA-based markers provide powerful tools for discerning variations within crop germplasm and for studying evolutionary relationships (Gepts *et al*., 1993).

RAPD have been used to construct genetic maps and for the molecular tagging of various agronomic traits in various crop species (O' brien, 1990; Williams *et al.,* 1993). It permits the identification of texa and the determination of phylogenetic relationship and intra-specific diversity at a molecular genetics level (Williams *et al.*, 1990).

2.2. RAPD markers in genetic diversity analysis of brinjal

Genetic diversity usually refers to the variation or differences between organisms at the DNA sequence level. This can be affected by natural or artificial (i.e. human) selection, mutation, recombination and other mechanisms. This genetic diversity can be successfully analyzed by using RAPD markers. Knowledge about germplasm diversity and genetic relationship among breeding materials are highly valuable tools in currently available for analysis of genetic diversity in germplasm accessions, breeding lines and populations. Literatures so far reviewed in context of analyzing molecular diversity of different brinjal varieties are described below:

Islam *et al.* (2014) investigated the genetic diversity in brinjal (*Solanum melongena* L.) using random amplified polymorphic DNA (RAPD). Fifteen brinjal germplasm and three decamer primers were used for random polymorphic DNA assay. A total of 17 fragments were obtained, out of which 12 (70.59%) were polymorphic. Each primer generated 4 to 8

amplified fragments with an average of 5.67 fragments per primer. The highest genetic distance (0.8873) and the lowest genetic identity (0.4118) were observed in Laffa (Elongated) versus Jessore L and Dharola combinations. The lowest genetic distance (0.1525) was observed in several cultivars. The unweighted pair-group method of arithmetic means (UPGMA) dendrogram was constructed from genetic distance and all brinjal cultivars were grouped into five clusters. The genetic diversity of brinjal cultivars reported in this study will be useful when planning future crosses amongst these cultivars.

Thakkar *et al.* (2014) analyzed 10 brinjal genotypes for their genetic diversity study through nineteen RAPD and ten ISSR primers, among which ten RAPD and six ISSR primers gave good polymorphism with 83.43 and 81.03 percent average polymorphism, respectively. The RAPD primers produce 480 amplified products with 71 bands among which 57 were polymorphic, giving average PIC and resolving power value of 0.837 and 3.48, respectively. The ISSR primers produced 52.17 average numbers of amplified products with 6.33 and 7.83 average number of polymorphic and total numbers of bands. The ISSR primers gave average PIC and resolving power value of 8.44 and 2.83, respectively. The RAPD primers produced band size ranged from 150-2659 bp while ISSR produced 113-3744 bp. Jaccard's similarity range for RAPD, ISSR and pooled data were 0.50 to 0.77, 0.55 to 0.86 and 0.52 to 0.75 respectively. Different combinations of similarity matrix and cophectic values of RAPD, ISSR and pooled data showed good correction through mantel's test (r=0.799 to 0.881) except for ISSR (r=0.486 to 0.463). The results indicated RAPD as a better marker and helped to study relatedness of different genotypes for future developments.

Mishra *et al*. (2014) studied to assess genetic diversity among diverse genotypes eggplant using RAPD technique. Twenty RAPD primers were used to amplify genomic DNA from 24 brinjal genotypes. Out of 20 primers, only 5 primers (6.5%) showed polymorphism and remaining 15 showed monomorphism. The DNA fragments of various sizes ranging from 500 bp to

1300 bp were amplified in various eggplant genotypes. Genetic distance (GD) ranged from 0.27 to 1.05. Cluster analysis showed that genotypes were clustered in two groups A and B on the basis of distinct and significant genotypes. The UPGMA dendrogram based on genetic distance segregated the 24 genotypes into six clusters. Thus the genotypes included in the diverse clusters could be used as a parent for hybridization in order to obtain a high heterotic response and better segregates in eggplant. Therefore, identification of genetically distinct varieties using RAPD marker could be a potential tool for eggplant improvement.

Ansari *et al.* (2013) assessed the genetic variability in the existing land races and primitive varieties, which are very much important for breeding programme of brinjal Multi-locus analysis using PCR based marker, Random Amplified Polymorphic DNA (RAPD) was used to assess the genetic diversity of some of the germplasm of *Solanum* species. It was observed that random primers (RAPD) showed polymorphism in revealing genetic polymorphism in *S. melongena* and *S. aethiopicum*. Hence, it may be considered as an efficient marker technique for genetic diversity study among *Solanum* species. Furthermore, genetic similarity reveals variability within the *S. melongena* whereas, wide range of dissimilarity between *S. melongen*a and *S. aethiopicum*.

Verma *et al.* (2012) conducted an experiment in which 11 RAPD and 6 SSR primers were used to analyze the genetic variation in 29 popular Indian brinjal varieties. The 11 RAPD primers generated 64 polymorphic bands with an average of 5.81 polymorphic bands per primer. Genetic distance based on RAPD markers among all the varieties ranged from 0.07 to 0.78 with an average of 0.33. All the 6 SSR primer pairs were polymorphic with a total of 25 detected alleles. The number of alleles per primer ranged from 2 to 10, with a mean of 4.67. UPGMA clustering for RAPD and SSR markers grouped all the brinjal varieties into two clusters, but grouping patterns were different for each of the marker system. However, majority of the cultivated varieties did not cluster concordant to the collection site information or phenotypic data

such as fruit shape or any other known traits. The genetic diversity of brinjal varieties reported in that study would be useful when planning future crosses amongst these varieties.

Ali *et al*. (2011) performed a study to analyze the genetic diversity of eggplant using inter-simple sequence repeat (ISSR) and RAPD procedures to subdivide 143 Chinese-cultivated eggplants based on coefficient of parentage, genetic diversity index (GDI) and canonical discriminant analysis. ISSR markers were more effective than RAPD markers for detecting genetic diversity, which ranged from 0.10-0.51, slightly lower than what is known from other crops. Their ISSR/RAPD data provide molecular evidence that coincided with morphological-based classification into three varieties and further subdivision into eight groups, except for two groups. Intensive use of elite parents and extensive crossing within groups have resulted in increased coefficient of parentage and proportional contribution but decreased GDI during the past decades. The mean coefficient of parentage and proportional contribution increased from 0.05 to 0.10% and from 3.22 to 6.46% during 1980-1991 and 1992-2003, respectively. The GDI of landraces was 0.21, higher than the 0.09 and 0.08 calculated for the hybrid cultivars released during the two periods. The recent introduction of alien genotypes into eggplant breeding programs may broaden the genetic base.

Jin *et al.* (2007) observed the genetic diversity among 53 aubergine germplasms by random amplified polymorphic DNA. Out of 80 random primers, 9 polymorphic primers were screened out and 81 bands were produced. Of them, 46 bands (56.79%) were polymorphic. The genetic similarity indices of all the germplasms varied from 0.72 to 0.99, suggested that the genetic diversity among germplasms was narrow. The dendrogram constructed using the UPGMA analysis suggested that 53 germplasms could be divided into 5 groups. The results showed that the classification did not fully correspond to the morphological characters and not associated with geological distribution.

Singh *et al.* (2005) studied genetic diversity and species relationships among 28 accessions of eggplant representing five species using Random amplified polymorphic DNA (RAPD) technique as a tool. A total of 144 polymorphic amplified products were obtained from 14 decamer primers, which discriminated all the accessions. The value of Jaccard's coefficient ranged from 0.05 to 0.82. The similarity result indicated the presence of high level of genetic diversity in eggplants and a dendrogram constructed by UPGMA method showed that *S. incanum* is closest to *S. melongena* followed by *S. nigrum*. Only one accession of *S. nigrum* and *S. surattense* was taken in the present study that showed grouping with each other. Genetically distinct genotypes identified using RAPD markers could be potential sources of germplasm for eggplant improvement.

Sadder *et al*. (2006) assessed genetic, morphological and agronomical variations for 10 Jordanian eggplant landraces that were grown by local framers and had been kept for many generations in addition to three cultivars. For morphological and agronomical traits, these eggplant landraces were evaluated and characterized under Jordan Valley conditions, where high temperature and salty soils prevail. Considerable differences in plant growth habit, yield and fruit characteristics were observed among the genotypes. These landraces can be used by breeders in breeding programs according to their objectives (yield under stress, bitterness, color, size, etc.). To assess genetic variation, the non-transcribed spacer of the 5S rDNA yielded invariant banding profiles for all genotypes. However, nine RAPD primers showed polymorphisms in 81 of 85 bands amplified. To assess genotype similarities, a total of 1053 data entries were utilized using Jaccard's similarity coefficient, and a UPGMA dendrogram was constructed from similarity values. The studied landraces showed considerable genetic variation among each other. The maximum similarity was recorded 80.5% while the most distant genotypes were having 4.8% similarity. One genotype did not cluster with other accessions, while the remaining accessions clustered into two major clusters:

cluster I with 10 genotypes and cluster II with two genotypes. Primers C02 and RAPD02 could differentiate all genotypes based on the banding profiles they generated (discrimination power of 1). However, other primers need to be combined to discriminate all genotypes from each other. The best, but not the only, combination between primers other than C02 and RAPD02 was the combination of C01, C15, and RAPD03. However, for future breeding projects, the detected morphological and agronomical variations need to be linked to genetic polymorphism through DNA markers.

2.3 Brinjal molecular characterization by RAPD

Demir *et al.* (2010) carried out an experiment on the molecular characterization of eggplant genotypes collected from different geographical regions of Turkey using SSR and RAPD markers. With amplification of five SSR loci, the number of alleles per microsatellite locus ranged from 2 to 10, with a total of 24 alleles. The greatest number of alleles was found at the emf21H22 locus (10 alleles); followed by emh11O01 and emf21C11 as five and four alleles, respectively. The average number of alleles per locus was 4.8. Using 11 decamer RAPD primers, 100 bands were amplified, among which 29 were polymorphic. The number of bands per primer ranged from seven (OPH10, OPH19, OPH20, OPH03) to 14 (OPB07). Primer OPB07 was the most polymorphic, generating 64% polymorphic bands; the rest of the primers gave less than 50% polymorphism. UPGMA dendrograms were used to examine the genetic relatedness of the genotypes.

Tiwari *et al*. (2009) carried out an experiment on molecular characterization of 19 advanced cultivars and landraces of brinjal using RAPD and ISSR markers. 29 RAPD primers generated a total of 240 amplified fragments, while 23 anchored and non-anchored ISSR primers produced 299 fragments. Of these, 66 (27.5%) RAPD and 56 (18.73%) ISSR fragments were polymorphic. All the cultivars could be distinguished based on RAPD

and/or ISSR profiles. A set of two RAPD primers, OPW 11 and OPX 07, was adequate to distinguish all the 19 cultivars. On the other hand, a minimum of ten ISSR primers were required to achieve the same result. Eleven cultivars could be identified by the unique presence or absence of one to four markers. The correlation between primer Rp and the number of cultivars distinguished by RAPD was r = 0.873, while that for ISSR it was r = 0.327. The correlation between PIC of primer and the number of cultivars distinguished was r = 0.324 for RAPD, while for ISSR primers it was r = -0.066. The UPGMA analysis grouped the cultivars into three main clusters with significant bootstrap support. While the cultivars bred at Indian Agricultural Research Institute, New Delhi formed one sub-cluster; others did not show a prominent region-based clustering.

Koundal *et al.* (2006) characterized 38 brinjal accessions including one wild-species; *Solanum sisymbrifolium* using random amplified polymorphic DNA (RAP D) and amplified fragment length polymorphism (AFLP) techniques. Out of 45 primers employed to generate RAPD profiles, reproducible patterns were obtained with 32 primers and 30 (93.7%) of these detected polymorphism. A total of 149 bands were obtained, out of which 108 (72.4%) were polymorphic. AFLP analysis was carried out using four primer combinations. Each of these primers was highly polymorphic. Out of 253 fragments amplified from these four primer combinations, 237 (93.6%) were polymorphic. The extent of pair-wise similarity ranged from 0.264 to 0.946 with a mean of 0.787 in RAPD, in contrast to a range of 0.103 to 0.847 with a mean of 0.434 in AFLP. The wild species clustered separately from the cultivated brinjal genotypes. In the dendrogram constructed separately using RAPD and AFLP markers, the brinjal genotypes were grouped into clusters and sub-clusters, and the varieties released by IARI remained together on both the dendrograms. All the 30 RAPD primers in combination and each of the four primer pairs in AFLP could distinguish the brinjal accessions from each other. AFLP was thus found to be

more efficient than RAPD in estimation of genetic diversity and differentiation of varieties in brinjal.

Karihaloo *et al.* (1995) carried out an experiment of RAPD analysis on 52 accessions of eggplant (*Solanum melongena* L.) and related weedy forms known as "insanum". 22 primers amplified 130 fragments. *Solanum melongena* exhibited 117 of the fragments, all of which were also present in insanum. Insanum displayed an additional 13 fragments not found in *S. melongena*. Overall, the insanum accessions were more diverse than those of *S. melongena*. The calculated similarity between them was 0.947. The RAPD results were closely concordant with the results of an electrophoretic isozyme survey performed on the same accessions. The concordance of the results shows that even though *S. melongena* and insanum are highly diverse morphologically, it is no longer appropriate to distinguish them genetically.

2.4. Genetic relationship and phylogenetic study of brinjal

Sifau *et al*. (2014) investigated four highly polymorphic random amplified polymorphic DNA primers to describe both the genetic relatedness and variability among 25 accessions of eggplant from Southwestern Nigeria. At a truncated line of 65%, five clusters and two ungrouped samples are distinguishable from the dendrogram. The data reveals that *Solanum dasyphyllum* Schum. & Thonn. is more closely related to *Solanum macrocarpon* L. than to *Solanum melongena* L. The relatedness between *Solanum incanum* L. and *Solanum melongena*, a probability of being progenitors from a common ancestral lineage was also shown. Occurrence of *Solanum scabrum* L. and *Solanum nigrum* L. in the same clusters different from *S. melongena*, is an indication of distant relatedness to *S. melongena* but close relatedness between them. High level of polymorphism was observed in this study going by the coefficient of variation, which exhibited a good separation from a conserved region of the genome. This study, therefore, reveals a wide and diverse genetic base in Nigerian eggplant (*Solanum*).

Sharmin *et al.* (2011) conducted an experiment to determine the genetic variation and relationships among three parents (BAU Begun-1, Dohazari G and Laffa S) and five F5 offsprings (BAU Begun-1 x Laffa S and BAU Begun-1 x Dohazari G) of eggplant using 3 RAPD decamer primers. In this study three primers generated 28 and 31 bands in Laffa S x BAU Begun-1 and Dohazari G x BAU Begun-1 respectively from which 16 (57.14%) and 18 (58.06%) were polymorphic, respectively. The co-efficient of gene differentiation (Gst) was 0.4534 reflecting the existence of high level of genetic variations among the genotypes. The dendrogram (UPGMA) constructed from Nei's genetic distance produced 2 main clusters of the parents and F5 offsprings. The result revealed that, genetic variation and relatedness to disease reaction as assessed using RAPD markers could be potential sources for the development of *Phomopsis vexans* resistant variety which is one of the major biotic stresses in eggplant in Bangladesh.

Biswas *et al*. (2009) studied genetic relationship among ten eggplant varieties using RAPD markers. Out of 21 primers screened four were selected. With these primers 76 clear and bright fragments were obtained of which 44 fragments considered polymorphic. The proportion of polymorphic loci and gene diversity values across all loci were 57.89% and 0.23, respectively. The UPGMA dendrogram based on genetic distance segregated the 10 varieties of eggplant into two main clusters. Dohazari, Kazla, Nayantara and ISD-006 were grouped together in cluster I whereas Uttara, Islampuri, Khatkhatia, Singnath, BARI Begun-08 and Eggplant Line-083 into cluster II. Kazla and Nayantara variety pair was very close to each other with the highest intervarietal similarity index (92.54%) and lowest genetic distance (0.14). On the other hand, Khatkhatia and Nayantara pair showed the lowest intervarietal similarity index (41.67%) with highest genetic distance (0.48). Therefore, identification of genetically distinct varieties using RAPD markers could be a potential tool for eggplant improvement.

2.5. RAPD Marker for disease resistance and crop improvement

Kumchai *et al.* (2013) conducted an experiment in which interspecific hybrids between cultivars of eggplant (*Solanum melongena* L.) and its wild relative *S. torvum*, which has disease resistance and desirable traits for crop improvement, were obtained by cross-hybridization and embryo rescue. Twenty-one hybrid progenies were obtained and examined based on morphological traits, RAPD and ISSR markers. Five of them were confirmed to be true interspecific hybrids. Eighteen and 14 bands from 7 RAPD and 14 ISSR primers, respectively, were polymorphic and present in all five hybrid seedlings and their parents. The morphological characteristics of leaf margin, inflorescence type and spine positions of the five seedlings were intermediate to the parents. These interspecific hybrids had low pollen viability, probably due to abnormal meiosis.

Laila *et al.* (2012) conducted an experiment in which F1 plants derived from crossing between a collar rot resistant eggplant varieties, BAU Begun 2, with four commercial cultivars, namely: BAU Begun 1, Laffa S, Dohazari G and ISD 006, were assessed for genetic variation and relationships among 5 parents and their F1 offspring, using Random Amplified Polymorphic DNA (RAPD) technique. Amplification with four decamer primers produced 20 RAPD bands of which 16 (80%) were polymorphic and 4 (20%) were monomorphic. The F1 progenies showed different gene diversity. The highest intra-variety similarity indices (Si) value were found in BAU Begun 2, Laffa S, BAU Begun 2×BAU Begun 1, BAU Begun 2×Laffa S -2, BAU Begun 2×Dohazari G -5 and BAU Begun 2×ISD 006 -2 (100%) The co-efficient of gene differentiation (Gst) was 0.88 reflecting the existence of high level of genetic variations among the genotypes. The dendrogram constructed from Nei's (1972) genetic distance produces two main clusters. Comparatively higher genetic distance (0.77) and lower genetic identity (0.46) was observed between the genotype combinations. The high genetic advance (94) for number of seeds per fruit indicates a very high probability of inheritance of resistance trait by F1 offspring from donor

parent BAU Begun 2. However, the cluster in dendogram (UPGMA) did not clearly reflect grouping based on the donor parent's character of resistance trait.

Baysal *et al.* (2010) *used* ISSR and RAPD markers to characterize *Fusarium oxysporum* f. *melongenae* isolates collected from eggplant fields in southern Turkey. Those isolates were not pathogenic to tomato. Pathogens were identified by their morphology, and their identity was confirmed by PCR amplification using the specific primer PF02-3. The isolates were classified into groups on the basis of ISSR and RAPD fingerprints, which showed a level of genetic specificity and diversity not previously identified in *Fusarium oxysporum f. melongenae,* suggesting that genetic differences are related to the pathogen in the Mediterranean region. The primers selected to characterize *Fusarium oxysporum* f. *melongenae* may be used to determine genetic differences and pathogen virulence. This study is the first to characterize eggplant *F. oxysporum* species using ISSR and RAPD.

Mutlu *et al.* (2008) carried out an experiment on Fusarium wilt (*Fusarium oxysporum* Schlecht. f. sp. *melongenae*), a vascular disease of eggplant (*Solanum melongena* L.) with the objectives of (1) to confirm the monogenic inheritance of fusarium wilt resistance in eggplant, (2) to identify molecular markers linked to this resistance, and (3) to develop SCAR markers from most informative markers. They reported the tagging of the gene for resistance to fusarium wilt (FOM) in eggplant using SRAP, RGA, SRAP-RGA and RAPD markers. Analysis of segregation data confirmed the monogenic inheritance of resistance. DNA from F_2 and BC_1 populations of eggplant segregating for *Fusarium* wilt resistance was screened with large number of primer combinations to detect polymorphism. Three markers were linked within 2.6 cM of the gene. The codominant SRAP marker Me8/Em5 and dominant SRAP-RGA marker Em12/GLPL2 were tightly linked to each other and mapped 1.2 cM from the resistance gene, whereas RAPD marker H12 mapped 2.6 cM from the gene and on the same side as the other two markers. The SRAP marker was

converted into two dominant SCAR markers that were confirmed to be linked to the resistance gene in the F_2, BC_1 and F_2 of BC_3 generations of the same cross. These markers provide a starting point for mapping the eggplant FOM resistance gene in eggplant and for exploring the synteny between solanaceous crops for fusarium wilt resistance genes. The SCAR markers will be useful for identifying fusarium wilt-resistant genotypes in marker-assisted selection breeding programs using segregating progenies of the resistant eggplant progenitor used in this study.

Sunseri *et al.* (2003) conducted an experiment in which inter specific hybrids were obtained by using an accession of *S. sodomeum* from Sicily and the eggplant variety Buia. The results of the screenings for eggplant genotypes tolerant/resistant to *Verticillium* wilt by using backcrosses were reported. In a naturally infected field, from 1998 to 2001, the resistance to *V. dahliae* of backcrossed progenies was increased by about 60%. The interspecific hybrids were both selfed and backcrossed using different types of eggplant. An integrated linkage map of eggplant (*Solanum melongena* L.) has been obtained by using 48 plants of an F_2 population. Starting from a genetic RAPDs map with ~ 100 markers mapped on 13 linkage groups, 4 AFLPs primer combinations were screened in order to improve the genetic map with the aim to achieve markers linked to *Verticillium* tolerance. Mendelian segregation of loci was verified by chi-square tests of the expected 3:1 and 1:1 ratios. Marker order was determined and all the data were combined to construct the most likely map. The integrated analysis of markers resulted in the construction of a map consisting of 273 loci and 12/13 linkage groups spanning 736 cM in a total. Linkage between different AFLP markers and the tolerance to *Verticillium* is discussed.

Nunome *et al.* (2001) constructed a linkage map of eggplant (*Solanum melongena* L.) using an F_2 population derived from a cross between a breeding line, EPL-1 and an introduced line, WCGR112-8, from India. The two parental lines showed contrasting responses to several pathogens and differences in

several morphological traits. Parental lines were screened with a large number of random primers for RAPD and 64 primer combinations for AFLP. The linkage map consisted of 181 loci, comprising 88 RAPD and 93 AFLP markers. These markers identified 21 linkage groups spanning 779.2 cM with an average distance of 4.9 cM. The linkage groups ranged from 1.9 to 95.6 cM in length and included 2 to 32 markers, respectively. The fruit shape and color development trait were scored and the linkage to the markers was investigated. The fruit shape showed a significant association with markers on linkage group 2. Color development in fruit, stem and calyx showed a significant association with markers on linkage group 7. These markers may provide valuable information for eggplant breeding.

2.6 Characterization of brinjal using other molecular markers

Ansari *et al.* (2013) studied the genetic diversity among 14 genotypes of brinjal using simple sequence repeat (SSR) markers. A total of 14 polymorphic SSR primer pairs were used. Amplification of genomic DNA of 14 genotypes yielded 50 fragments, of which 43 were polymorphic. A clear cut differentiation was exhibited among the genotypes. The range of similarity coefficient varied from 17.8% (between *S. aethiopicum* L. and *S. melongena* L.) to 94.1% (between PB-71 and NDB-1) followed by 88.9% (between SMB-115 and KS-331) and 88.6% (between BARI and PB-67). SAHN cluster analysis using UPGMA method separated the genotypes into six cluster groups. *Solanum aethiopicum* and PB-67 were positioned as single genotype in separate groups i.e., cluster-I and II, SMB-115 and KS-331 in cluster-III, BARI, PB-66 and Pant Rituraj in cluster-IV, WB-1, PB-4, PB-70 and LC-7 in cluster-V and PB-71, Pant Samrat and NDB-1 in cluster-VI. Morphological characters *viz*., shape, size and peel colour of brinjal fruits and plant type showed a positive relationship with the DNA based molecular analysis through SSR markers.

Ge *et al.* (2013) examined the genetic diversity and relationships among 92 eggplant accessions collected from seven areas in China using one hundred simple sequence repeat (SSR) markers. Analysis of these SSRs revealed a moderate amount of polymorphism with an average polymorphism information content (PIC) value of 0.285. The average value of number of effective loci (Ne), expected heterozygosity (He) and Shannon's Information index (I) were 1.631, 0.323 and 0.570, respectively. The levels of genetic diversity observed in the seven areas were not evenly distributed and decreased from south to north. The F-statistics for these populations showed medium genetic differentiation (Fst = 0.129) and considerable gene flow (Nm = 2.230). The neighbor-joining (NJ) cluster analysis divided the seven areas into four clades that largely corresponded to geographic divisions. Population structure analysis resulted in two subgroups that generally fit with previous classification scheme. The results will be useful for eggplant germplasm management and will lead to more efficient use of germplasm in eggplant breeding.

Adeniji *et al.* (2012) investigated seven *Solanum* species (eggplants) for molecular diversity. 39 *Solanum* accessions, a landrace and tomato variety (LBR 48) were molecularly analyzed by simple sequence repeat (SSR) marker technique. A dendrogram was obtained based on the Jaccard’s coefficient of similarity and unweighted pair group method with arithmetic mean (UPGMA) clustering. A total of 417 alleles were amplified with the number of alleles ranging from 5 (EM 141) to 38 (EM 120 b). Polymorphism was fairly high (0.05 to 0.92) among SSR markers with high number of repeats. Findings indicated that entries originating from different parts of the world did not form a distinct cluster, and there was no association between SSR marker pattern and geographical origin. SSR markers indicated a strong genetic affinity among *S. viarum, S. melongena* and *S. aethiopicum* group. Genetic relatedness between *S. dasyphyllum* and *S. macrocarpon* and between *S. aethiopicum* and *S. macrocarpon* are important for breeding. SSR markers assayed were informative for phylogenetic analysis; and have the potential to serve as perfect

markers for studying variation. For plant breeders, close genetic relationships detected provide an avenue for introgression of high yielding and resistant genes into commercial and farmers' varieties.

Tumbilen *et al.* (2011) analyzed genetic diversity in Turkish eggplant using AFLP markers. In their study, the genetic variability of 67 Turkish eggplant accessions from the national germplasm collection was assessed with 30 morphological traits and AFLP markers. Morphological analysis indicated considerable variability especially for semi-long and round types. For molecular characterization, accessions of *S. macrocarpon*, *S. aethiopicum* and *S. linnaeanum* were included as outgroups. Ten primer combinations were used and yielded 488 polymorphic fragments with PIC values ranging from 0.03 to 0.50. Of the polymorphic fragments, 144 (29%) were specific to *S. melongena* accessions while 73, 49 and 16 fragments were specific to *S. macrocarpon*, *S. aethiopicum* and *S. linnaeanum*, respectively. UPGMA cluster analysis of the AFLP data resulted in a dendrogram, which had a very high correlation (r=0.97) with the similarity matrix data. Genetic similarity in the dendrogram ranged from 0.30 to 0.95 with the related *Solanum* species located outside the *S. melongena* clusters, as expected. Genetic similarity of the *S. melongena* accessions ranged from 0.68 to 0.95 indicating good genetic diversity present in the Turkish national collection.

Li *et al.* (2010) employed sequence-related amplified polymorphism (SRAP) markers to evaluate genetic variation in a diverse collection of 56 *Solanum* accessions. Fifty-five SRAP primer combinations were used and a total of 635 polymorphic bands were observed. Cluster analysis by the unweighted pair-group method with arithmetic averages based on similarity matrices indicated that there were three clusters: (i) *S. melongena*; (ii) *S. aethiopicum*; (iii) *S. surattense*. The coefficients of genetic similarity among all the accessions ranged from 0.04 to 0.96 with an average of 0.73, and averaged 0.78 among *S. melongena* accessions originated from China, indicating extensive genetic

variation. These results demonstrated that SRAP can be efficiently used to estimate genetic diversity and analyze phylogenetic relationship.

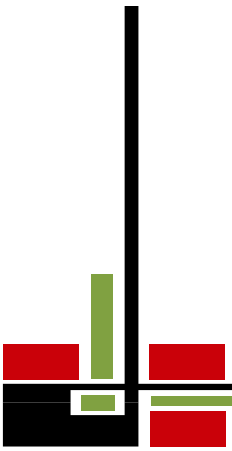
Khorsheduzzaman *et al.* (2008) characterized five brinjal (*Solanum melongena* L.) genotypes using Simple Sequence Repeats (SSR) markers. All the genotypes showed considerable variation in respect of morphological, anatomical and biochemical aspects. For study of relatedness, plant genomic DNA was extracted by CTAB based method using 11 randomly selected primers produced from Calgene Inc. USA. The primers developed 22 bands through PCR amplification out of which 15 from 3 primers and were polymorphic. Genetic similarities of SSR profiles were estimated based on Jaccard's coefficient value. The dendrogram generated two clusters and they were clearly distinct and separated from each other. Cluster-I consisted of genotypes TURBO and BL009. Where cluster-II comprised of genotypes EG058, EG075 and ISD006. Genotype TURBO and BL009 were identified as the diverse genotype and showed maximum 17% dissimilarity from EG058, EG075 and ISD006. The similarity value ranged from 0.83 to 1.00 which indicated the presence of narrow range of genetic diversity at molecular level but have still a possibility of crossing among the genotypes of two clusters. The banding pattern of different genotypes could be utilized as reference for further comparisons.

Nunome *et al.* (2008) evaluated the potential of microsatellite markers for use in genetic studies of eggplant (*Solanum melongena* L.). A genomic library of eggplant was screened for GA and GT repeat motifs to isolate microsatellite clones. The frequency of each repeat motif in the eggplant genome was found to be every 3200 kb for GA repeats and every 820 kb for GT repeats. 61 percent of GT repeats were found to directly flank AT repeats. A total of 37 polymerase chain reaction (PCR) primer pairs were designed, 23 of which amplified a single product or several products. The level of microsatellite polymorphism was evaluated by using *S. melongena* lines and related *Solanum* species. Two to six alleles per primer pair were displayed in the *S.*

melongena lines and 2 to 13 alleles were displayed in the *Solanum* relatives. Seven microsatellites showed polymorphism between parental lines of the mapping population and segregated in a co-dominant Mendelian manner. These microsatellite loci were distributed throughout the linkage map.

Furini *et al.* (2004) conducted an experiment in which, a total of 94 *Solanum* accessions, including eggplants and related species, were morphologically characterized based on greenhouse observations, and molecularly analyzed by the AFLP technique. Morphological parameters were helpful in assessing similarities or differences among accessions, and molecular data were used to support morphological conclusions. A dendrogram was computed based on the Dice genetic distances using the neighbour-joining method. The analysis was efficient in the assignment of a species name for eight out of nine accessions that were not previously classified, and revealed that 14 further accessions were misnamed in the collection originally received. The AFLP technique was revealed as an efficient tool in determining genetic relationships among species. In general, morphological observations were consistent with molecular data, indicating that both approaches complemented to define the phylogenetic status of a large genus like *Solanum.* In terms of eggplant breeding, the molecular analysis of the *Melongena* complex, and of the other sections of the subgenus *Leptostemonum,* established useful germplasm relationships in the gene pool available for the genetic improvement of the cultivated species.

From the above mentioned review of literature, it was clearly found that RAPD analysis is very efficient in molecular genetic variation studies within and/or among the populations of brinjal cultivars or wild species. The present study is aimed to determine genetic variation and relatedness between eleven varieties of brinjal of Bangladesh using RAPD markers. So that the level of genetic distance and similarities between the varieties may be estimated which can help in selective breeding techniques for variety improvement using that eleven varieties.



Chapter III

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

The chapter focused on the materials and methods of the experiment. The details of the methodology followed for the study have been described in this chapter as follows:

3.1 Experimental site and time duration

To achieve the objectives, the experiment was carried out during the period from October 2013 to May 2014 at the Biotechnology Laboratory, Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207.

3.2 Study materials

The plant material for this study consists of 11 varieties of brinjal *viz*. BARI begun 1, BARI begun 4, BARI begun 5, BARI begun 6, BARI begun 7, BARI begun 8, BARI begun 9, BARI begun 10, BARI hybrid begun 1, BARI hybrid begun 3 and BARI hybrid begun 4.

All the varieties were collected from Horticulture Research Centre (HRC) of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

3.3 Collection of leaf sample

Soil was collected from nearby nursery for the germination of seed. Seed germination was performed in germination tray with proper labeling. Seeds were sown on November 6, 2013. Seedlings were germinated within a week.

In order to carry out RAPD analysis, fresh and young leaves from the seedlings of about 30 days old of each genotype were collected and used as the source of genomic DNA.

3.4 Extraction of genomic DNA

Genomic DNA was extracted from the leaf sample using appropriate protocol of Phenol-Chloroform-Isoamyl alcohol method described by Islam *et al.* (2013) with some modifications. The following reagents and methods were used for the isolation of total genomic DNA.

3.4.1 Reagents used

1. Extraction buffer, pH= 8.0

   Composition of extraction buffer is 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 (Tris-EDTA) buffer, pH=8.0
   - 1M Tris-HCl
   - 0.5M EDTA
   - D.H_2O
4. Isopropanol
5. 0.3M Sodium Acetate
6. Absolute (100%) ethanol
7. Ethanol (70%)
8. RNAase
9. Ethidium Bromide solution.

3.4.2 Reagent Preparation for DNA Extraction (Stock Solution)

Extraction buffer (1000 ml)

- 100 ml 1M Tris HCl (p^H 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 dd.H_2O was added to make the volume upto 1000 ml.
- Then the mixture was mixed well and autoclaved.

1M Tris-HCl pH 8.0 (250 ml)

- At first 30.28 g Tris was taken in a volumetric flask (5000 ml)
- 100 ml dd.H_2O was added.
- pH was adjusted to 8.0 by adding HCl.
- Then sterilized dd.H2O was added to make the volume up to 250 ml.
- The solution was autoclaved.

0.5M EDTA. pH8.0 (250 ml)

- At first 46.53 g EDTA.$2H_2O$ was added in a volumetric flask (5000 ml)
- 100 ml dd.H_2O was added.
- Then 4 g NaOH was added.
- pH was adjusted to 8.0 with NaOH
- Then sterilized dd.H_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 dd.H_2O.
- It was then mixed well and autoclaved.

1% SDS (100 ml)

- 1g of SDS was added in 100 ml dd.H_2O in a 250 ml beaker.
- As SDS is hazardous, so the mixture was mixed by a hot top magnetic stirrer well but 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 EDTA (pH 8.0) was added.
- Sterilized dd.H_2O was added to make the volume up to 100 ml.

Composition of 5X TBE buffer (1 litter)

- 54 g Tris-HCl
- 27.5 g of Boric acid
- 4.65 g of EDTA
- pH= 8.3

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

- At first 50 ml Phenol was taken in a volumetric flask (250 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 dd.H_2O.
- Then it was dissolved completely with the help of necessary heat (at 50℃ in water bath for 30 minutes).

70% Ethanol (1000 ml)

- 700 ml absolute ethanol was mixed with 300 ml dd.H_2O

3.5 Methods for DNA extraction

The following steps were followed for DNA extraction.

For isolation of genomic DNA, young, soft, vigorous and actively growing fresh leaf tissues were collected from 11 brinjal varieties. Total DNA was isolated by using Phenol: Chloroform: Isoamayl alcohol and ethanol precipitation method. The youngest leaves were selected in order to make the tissue grinding process easy. The leaves were taken and washed consequently in sterile distilled water and ethanol. Then the leaves were dried on fresh tissue paper to remove spore of microorganisms or any other source of foreign DNA. Approximately 200 mg of young leaves were cut into small pieces and then taken in morter. 300 µl of extraction buffer were added and then leaf tissues were ground manually with the help of a pestle. The sample was then taken into eppendorf tube and 300 µl of extraction buffer was added to it. The ground sample was then vortexed for 20 seconds in a vortex mixture and then incubated at 65°C for 20 minutes in hot water bath. Equal volume (500 µl) of chloroform: Isoamyl alcohol (24:1) was added to the tube and then it was vortexed for 10 seconds. The solution was 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. 250 µl of Isopropanol (0.6 volume of the liquid) was added to it. It was then tapped by finger for 20-30 seconds. The genomic DNA was visible as cotton like structure. 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 500 μl of absolute (100%) cold ethanol plus 20ml 0.3M sodium acetate. It was shaken gently. 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. The DNA pellet was then air dried for 2-3 hours. It was then dissolved in an appropriate volume (30-40 µl) of TE buffer and treated with 3 µl of RNAase for removing RNA. Then it was spinned for 4-5 seconds.

Finally, the DNA samples were stored in freezer at -20°C.

Precautions

As the precautionary measures, all glassware, micropipette tips, micro centrifuge tubes, distilled water and buffer solutions were properly autoclaved to avoid contamination of DNA. Scissors, forceps etc. were sterilized with absolute ethanol.

As Ethidium Bromide (Et-Br) is a powerful mutagen and carcinogenic in nature, hand gloves were used when handling anything that had been exposed to Et-Br.

3.6 Confirmation of DNA preparation

Sometimes isolated genomic DNA contains a large amount of RNA and pigments, which usually cause over estimation of DNA concentration on a spectrophotometer. To confirm DNA preparation, 1% agarose gel was used for assessing the quality of the genomic DNA and the amount of RNA present.

3.6.1 Preparation of 1% agarose gel

Reagents:

1. Agarose powder
2. 5X TBE Buffer (pH 8.3)
3. Ethidium Bromide

Procedure

1 g of agarose powder was taken in a 500 ml Erlenmeyer flask containing 100 ml of electrophoresis buffer (1X TBE buffer) prepared by adding 20 ml of 5X TBE buffer in 80 ml of sterile de-ionized water. The flask was enclosed with aluminum foil paper to prevent excessive evaporation. The flask was heated into a microwave oven for about 3 minutes with occasional swirling to generate uniform suspension until no agarose particle was seen to generate

homogeneous and crystal clear suspension. The agarose solution was cooled to about 45-50°C (flask was cool enough to hold comfortably with bare hand) and 1µl ethidium bromide (DNA stain) was added and mixed well by gentle shaking to make the DNA visible under ultraviolet light box (Trans-illuminator). The molten gel was poured immediately on to a clean gel bed (15 x15 x 2 cm^3 in 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 30 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.

3.6.2 Preparation of DNA samples for electrophoresis

The samples were all in the same concentration in buffer. For each sample, 4 µl dd.H_2O and 3 µl loading dye (0.25% xylene ethanol, 0.25% bromophenol blue, 30% glycerol and 1mM EDTA) was 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. Finally, 3 µl extracted DNA was added to it and mixed well. The sample was then loaded into the well of the gel and allowed them to sink to the bottom of the wells.

The gel was placed in the electrophoresis chamber keeping the gel horizontal and submerged in 1X TBE buffer (running buffer). The final level of buffer was about 5 mm above the gel. The gel tank was covered and the electrophoresis power supply was connected and turned on to move DNA from negative to positive electrode (Black to Red) through the gel. Electrophoresis was carried out at 90V for about 45 minutes.

3.6.3 Documentation of the DNA samples

The gel was taken from the gel chamber and was placed on an ultraviolet light box (UV transilluminator) to examine and photographed by a Gel Cam

Polaroid camera. Better quality bands showing DNA samples were taken for quantification and working solution preparation.

3.7 Quantification of DNA

The concentration of DNA is one of the important variables for PCR amplification. As different DNA extraction methods produce DNA of widely different purity, it may be necessary to optimize the amount of DNA used in RAPD assay to achieve reproducibility and strong signal. Below a certain critical concentration of genomic DNA, RAPD amplification is no longer reproducible (Williams *et al*., 1993). So it is necessary to stay above this critical concentration. Moreover excessive DNA concentration is likely to produce poor resolution or "smears'' resulting in a lack of clearly defined bands in the gel. Therefore, quantification of DNA in each sample was determined spectrophotometrically.

Concentration of genomic DNA was examined by calculating the ratio of the optical density measured at 260 nm using a spectrophotometer and stored in freezer. After warming up the spectrophotometer UV lamp for about 20 minutes, the wavelength was set at 260 nm as it is the absorbance maxima for the nucleic acids. A square cuvette (the "zero" or "black" cuvette) was filled with 2 ml sterile distilled water and placed in the cuvette chamber and the absorbance reading was adjusted to zero for standardization. The best sample was prepared by taking 2 µl of each DNA sample in the cuvette containing 2 ml sterile distilled water and thorough mixing by pipetting. After recording the absorbance reading, the cuvette was rinsed out with sterile water, tamped out on a paper wipe, and absorbance reading for each sample was recorded in the same way. The absorbance readings of extracted DNA samples of 11 brinjal cultivars are listed in the table 4.

Using the absorbance readings, the original sample concentrations were determined according to the following formula:

$$\text{Concentration of DNA } (\mu L) = \text{Absorbance} \times \frac{\text{Volume of distilled water}}{\text{Amount of DNA } (\mu L)} \times \text{Conversion factor } (0.05) \times 100$$

Where,

Absorbance = Spectrophotometer reading

Volume of distilled Water = 2000 µl

Amount of DNA = 2 µl

Conversion factor = 0.05

3.8 Preparation of working solution of DNA samples

DNA concentrations were adjusted to 25 ng/µl for doing PCR using the following formula:

$$\mathbf{V_1 \times S_1 = V_2 \times S_2}$$

Where,

V_1= Initial volume of DNA solution (µl)

S_1= Initial DNA concentration (ng/µl)

V_2= Finial volume of DNA solution (µl)

S_2= Final DNA concentration (ng/µl)

3.9 Amplification of RAPD markers by PCR

3.9.1 Principle of the amplification of RAPD

For performing amplification of RAPD, a single oligonucleotide of arbitrary DNA sequence is mixed with genomic DNA in the presence of a thermo-stable DNA polymerase and a suitable buffer and then it is subjected to temperature cycling conditions typical to the Polymerase Chain Reaction (PCR). The products of the reaction depend on the sequence and length of the oligonucleotide, as well as the reaction conditions. At an appropriate annealing temperature the single primer binds to sites on opposite strands of the genomic DNA that are within an amplifiable distance of each other (e.g., within a few thousand nucleotides) and a discrete DNA segment is produced. The presence or absence of this specific product, although amplified with an arbitrary primer, will be diagnostic for the oligonucleotide binding sites on the genomic DNA. In practice, the DNA amplification reaction is repeated on a set of DNA samples with several different primers, under conditions that result in several amplified bands from each primer. Often a single primer can be used to identify several polymorphisms, each of which matches to a different locus.

3.9.2 List of RAPD Primer

Nine decamer RAPD primers *viz.* OPA-18, OPB-04, OPB-06, OPB-08, OPD-02, OPF-08, OPG-19, OPP-13, and OPW-08; (Operon Technologies, Inc. Alameda, California, USA.) were initially screened for PCR reaction on 11 brinjal varieties for their ability to produce polymorphic DNA band.

The list of RAPD primer and their sequences are given in table 1.

Table 1. Primer name, sequence and GC content of RAPD primers used for the detection of polymorphism in brinjal genotypes

Name of RAPD primer	Sequence of the primer (5´ to 3´)	(G+C) content %
OPA-18	AGGTGACCGT	60
OPB-04	GGACTGGAGT	60
OPB-06	TGCTCTGCCC	70
OPB-08	GTCCACACGG	70
OPD-02	GGACCCAACC	70
OPF-08	GGGATATCGG	60
OPG-19	GTCAGGGCAA	60
OPP-13	GGAGTGCCTC	70
OPW-08	GACTGCCTCT	60

3.9.3 PCR amplification

The amplification conditions were based on the reaction mixture described by Williams *et al.* (1990) with some modifications. PCR reactions were performed on each DNA sample in a 25 µl reaction mix containing 12.5 µl 2X Taq Master Mix (GeneON, Cat. No: S113), 7.5 µl of sterile de-ionize water and 2.5 µl of genomic DNA (25ng/µl). DNA amplification was performed in an oil-free thermal cycler (Esco Technologies Swift™ Mini Thermal Cyclers).

PCR reactions

PCR reactions were performed following two systems.

(1) Using PCR reaction reagent separately (each reagent is measured separately and a mixture was prepared) and

(2) Using 2X Taq Master Mix (GeneON, Germany)

3.9.3.1 Using individual PCR chemicals

PCR reactions were performed on each DNA sample in a 25 µL reaction mix containing 4 µL of 10x Ampli Taq polymerase buffer, 2.5 µL of 10 µM primer, 2 µL of 250 µM dNTPs, 1 µL of Ampli Taq DNA polymerase (Bioneer, Korea) and 4 µL (100 ng) of genomic DNA and rest amount of sterile deionized water. DNA amplification was performed in an oil-free thermal cycler (Esco Technologies Swift™ Mini Thermal Cyclers). The reaction mix was preheated for 4 mins at 94°C for pre-denaturation and then 33 cycles consisted of 1 min denaturation at 94°C, 50 sec at 36°C for annealing and 2 minutes at 72°C for elongation or extension. After the last cycle, a final step of 7 minutes at 72°C was added to allow complete extension of all amplified fragments.

Reaction mix preparation to perform Polymerase Chain Reaction (PCR)

William *et al.* (1990) mentioned the conditions for RAPD amplifications reactions with some modifications. PCR reactions were performed on each DNA sample in a 25 µL reaction mix containing following reagents:

Table 2. Reaction mixture composition for PCR

Reagents	Amount
Taq DNA polymerase buffer (10X)	4 µL
Primer (10 µM)	2.5 µL
dNTPs (250 µM)	2 µL
Taq DNA polymerase	1 µL
Genomic DNA (25 ng/µL)	4 µL
Sterile de-ionized water	11.5 µL
Total reaction volume	**25 µL**

From frozen stocks the PCR buffer, dNTPs, primer and DNA samples solutions were thawed, mixed by vortexing and kept on ice. DNA templates were pipetted first into PCR tubes compatible with the thermo cycler used (0.5 mL). A pre-mixture was then prepared in the course of the following order: reaction buffer, dNTPs, DNA template and sterile distilled water. Taq polymerase enzyme was then added to the pre-mixture. The pre-mixture was then mixed up well and aliquoted into the tubes that already containing primer. The tubes were then sealed and placed in a thermo cycle and the cycling was started immediately.

3.9.3.2 PCR reaction with 2X Taq Master Mix (Geneon, Germany)

Ready mix Taq DNA polymerase and other composition were used for PCR reaction. The ratio of each chemicals are given below:

Table 3: Ready mix PCR mixture for amplification of brinjal genotypes

Reagernts	Amount (µl)
2X Taq Master Mix	12.5
RAPD primer	2.5
Sterile de-ionize water	7.5
Genomic DNA (25 ng/µl)	2.5
Total reaction volume	**25**

From the frozen stocks the Master Mix, primer and DNA samples solutions were thawed, mixed by vortexing and kept on ice. DNA templates were pipetted first into PCR tubes compatible with the thermo cycler used. The required amount of 2X Taq Master Mix, RAPD primer and sterile de-ionized water were then added into it. The mixture was then mixed up well. The tubes were then sealed and placed in a thermo cycle and the cycling was started immediately.

3.9.4 Thermal Profile

DNA amplification was performed in an oil-free thermal cycler (Esco Technologies Swift™ Mini Thermal Cyclers). The PCR was programmed as per Biswas *et al*. (2009) used for brinjal DNA amplification. According to them the reaction mixture was pre-denatured at 95^{o} C for 5 minutes followed by 33 cycles of 45 seconds denaturation at 95^{o} C, 30 seconds annealing at 30 ^{o} C and elongation or extension at 72 ^{o} C for 1 minute. After the last cycle, a final step of 5 minutes at 72 ^{o} C was added to allow complete extension of all amplified fragments. After completion of cycling program, reactions were held at 4 ^{o} C.

3.9.5 Electrophoresis of the amplified products

From each sample PCR products were confirmed by running 2% agarose gel containing 0.5 µl ethidium bromide in 1X TBE buffer at 90V for 55 minutes. Loading dye (4.0 µl) was added to the PCR products and loaded in the wells. Two molecular weight markers 100 bp (BIONEER, Cat. No. D-1030 South Korea) and 1kb (BIONEER, Cat. No. D-1040, South Korea) DNA ladder were also loaded on left and right side of the gel respectively. Under Ultra Violet (UV) light on a trans-illuminator RAPD bands were observed and documented by taking photograph using a Gel Cam Polaroid camera.

3.9.6 Documentation of the DNA samples

After staining, the gel was taken out carefully from the gel chamber and was placed on high performance ultraviolet light box (UV trans-illuminator) of gel documentation for checking the DNA was observed as band and photographed by a Gel Cam Polaroid camera.

3.10 RAPD data analysis

Since RAPD markers are dominant, we assumed that each band represented the phenotype at a single allelic locus (Williams *et al.,* 1990). Two molecular weight markers, 1kb and 100 bp DNA ladder were used to estimate the size of the amplified products by comparing the distance travelled by each fragments with known sized fragments of molecular weight markers. All the distinct bands or fragments (RAPD markers) were thereby given identification numbers according to their position on gel and scored visually on the basis of their presence (1) or absence (0), separately for each individual and each primer.

The scores obtained using all primers in the RAPD analysis were then pooled to create a single data matrix. This was used to estimate polymorphic loci, Nei's (1972) gene diversity, Genetic distance (GD) and to construct a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among

populations using a computer program, POPGENE (Version 1.31) (Yeh *et al.*, 1999). The same program was used to perform test of homogeneity in different locus between population pairs.

Estimation of gene frequencies of RAPD loci was based on the assumption of a two-allele system. Of the two alleles, only one is capable of amplification of a RAPD band by primer annealing at an unknown genomic position (locus). The other is the 'null' allele incapable of amplification, mainly because of loss of the primer annealing site by mutation. The two-allele assumption was in most cases acceptable, because co-dominent loci showing band shifts are few (Elo *et al.*, 1997; Welsh and McClelland, 1990). In this system only a null homozygote is detectable as negative for the RAPD band of interest.

Under the assumption of Hardy-Weinberg equilibrium, the null allele frequency (q) may be $(N/n)^{½}$ where N and n are the number of band negative individuals observed and the sample size respectively. The frequency of the other allele is 1-q, the assumption of the two allele system enables us to calculate the Nei's genetic distance (Nei's, 1972) from the RAPD pattern.

Genetic similarity values defined as the fraction of shared bands between the RAPD profiles of any two individuals on same gel were calculated manually by RAPD markers of the molecular weight on the data matrix according to the following formula:

$$\textbf{Similarity index (SI)} = \frac{2Nxy}{Nx+Ny}$$

Where, N_{xy} is the number of RAPD bands shared by individuals x and y respectively, and N_x and N_y are the number of bands in individuals x and y respectively, (Chapco *et al.*, 1992, Wilde *et al.*, 1992, Lynch, 1990).

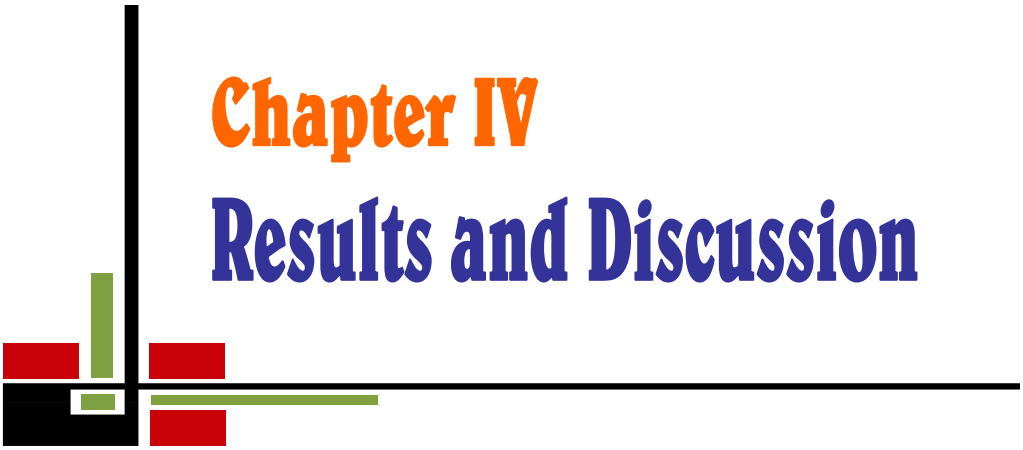
The SI value ranges from 0 to 1. When SI=1.0, the two DNA profiles are identical and when SI is 0.0, there are no common bands between the two profiles. Within population similarity (Si) was calculated as the average of SI across all possible comparisons between individuals within a population.

Between population similarity (Sij) was calculated as the average similarity between each paired individuals of population i and j (Lynch, 1991).

Nei's genetic distance and genetic identity values were computed from frequencies of polymorphic markers to estimate genetic relationship between the studied 11 brinjal germplasm using the Unweighted Pair Group Method of Arithmetic Means (UPGMA) (Sneath and Sokal, 1973). The dendrogram was constracted using a computer program, POPGENE; (Version 1.31) (Yeh *et al.,* 1999).

3.11 Precautions:

1. To avoid DNAase contamination, all glassware, micropipette tips, eppendorf tubes, glass pipettes, de-ionized water and buffer solutions were properly autoclaved. Scissors, forceps were sterilized with absolute ethanol.
2. Since Ethidium Bromide (Et-Br) is a powerful mutagen and carcinogenic in nature, hand gloves were used when handling anything that has been exposed to Et-Br.
3. Always power pack was kept turn off and the leads were unplugged before opening the electrophoresis unit to avoid electrical hazard.
4. A trans-illuminator produces UV radiation of 254 nm range. The weave length can cause eye damage. Thus eye protector was used while working with it.
5. The usual precautions were maintained when performing PCR reactions. All the disposables 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 during handling of PCR components. Contamination of PCR components was avoided.



Chapter IV
Results and Discussion

CHAPTER IV
RESULTS AND DISCUSSION

This chapter comprises the presentation and discussion of the results of the experiment. The results were obtained from the experiment using RAPD markers on brinjal varieties. In the RAPD analysis significant genetic variation and polymorphisms for characterization of different brinjal cultivars were identified. The results of the experiment were presented and expressed in Table 4 to 8, Figure 1 and Plate 1 to 8 for ease of understanding.

4.1 DNA confirmation and quantification

The extracted genomic DNA of 11 samples were loaded on 1% agarose gel for conformation and quantification of DNA solution. It revealed that, all the samples showed clear DNA band in each well (Figure 1.). There was no smear and sharing of DNA was also done in spectrophotometer. The DNA quantification results are presented in table 4. It revealed that maximum 3900 ng/µl genomic DNA was observed in the sample BARI begun 8 and minimum 2750 ng/µl in the sample BARI begun 1. It is recommended that, only 25-50 ng/µl DNA is required for PCR amplification. Hence, the genomic DNA of each sample was diluted on the basis of concentration. The working DNA sample was prepared for PCR works.

Table 4. Absorbance reading and concentrations of different DNA samples collected from 11 brinjal cultivars

Brinjal varieties	Serial No	Absorbance reading (260 nm)	DNA concentration (ng/µl)
BARI begun 1	1	0.055	2750
BARI begun 4	2	0.063	3150
BARI begun 5	3	0.059	2950
BARI begun 6	4	0.072	3600
BARI begun 7	5	0.067	3350
BARI begun 8	6	0.078	3900
BARI begun 9	7	0.075	3750
BARI begun 10	8	0.061	3050
BARI hybrid begun 1	9	0.057	2850
BARI hybrid begun 3	10	0.064	3200
BARI hybrid begun 4	11	0.067	3350

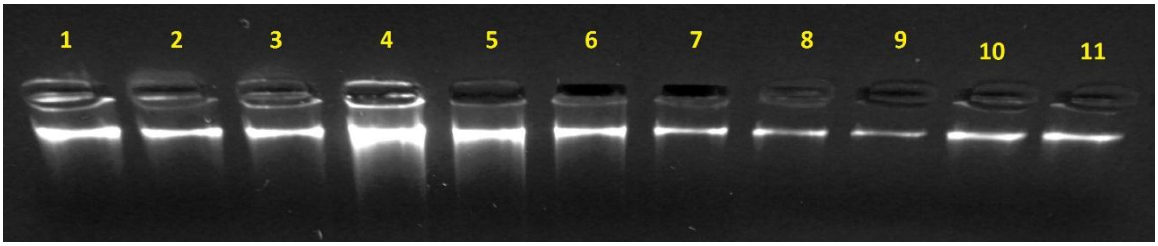


Plate 1. Confirmation of genomic DNA samples of 11 genotypes of brinjal.

(Lane 1: BARI begun 1, Lane 2: BARI begun 4, Lane 3: BARI begun 5, Lane 4: BARI begun 6, Lane 5: BARI begun 7, Lane 6: BARI begun 8, Lane 7: BARI begun 9, Lane 8: BARI begun 10, Lane 9: BARI hybrid begun 1, Lane 10: BARI hybrid begun 3, Lane 11: BARI hybrid begun 4)

4.2 RAPD Primer selection for PCR amplification

Nine primers (OPA-18, OPB-04, OPB-06, OPB-08, OPC-05, OPD-02, OPF-08, OPP-13 and OPW-08) were initially screened on 11 brinjal varieties for their ability to produce polymorphic patterns. Among them five primers *viz*. OPB-04, OPB-08, OPD-02, OPP-13 and OPW-08 gave reproducible and distinct polymorphic amplified products. Strong and weak bands were produced in the RAPD reactions. Weak bands result from low homology between the primer and the pairing site on the DNA strand (Thormann *et al.*, 1994). The present findings agree with those of Hardy *et al*. (1992) and Williams *et al*. (1993).

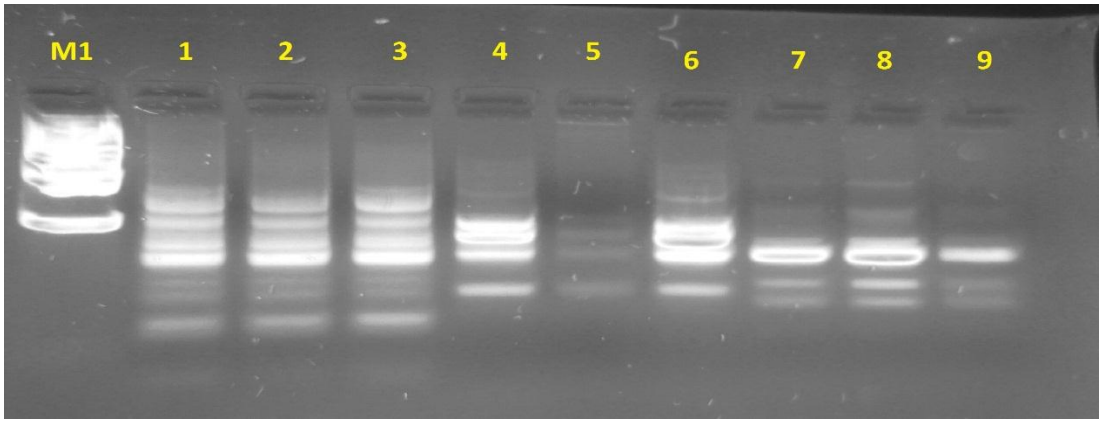


Plate 2. Primer test: PCR amplification products by 3 different decamer random primers using DNA from different brinjal varieties

(Lane 1-3: OPD-02; Lane 4-6: OPP-13; Lane 7-9: OPG-19. M1=1Kb DNA ladder)

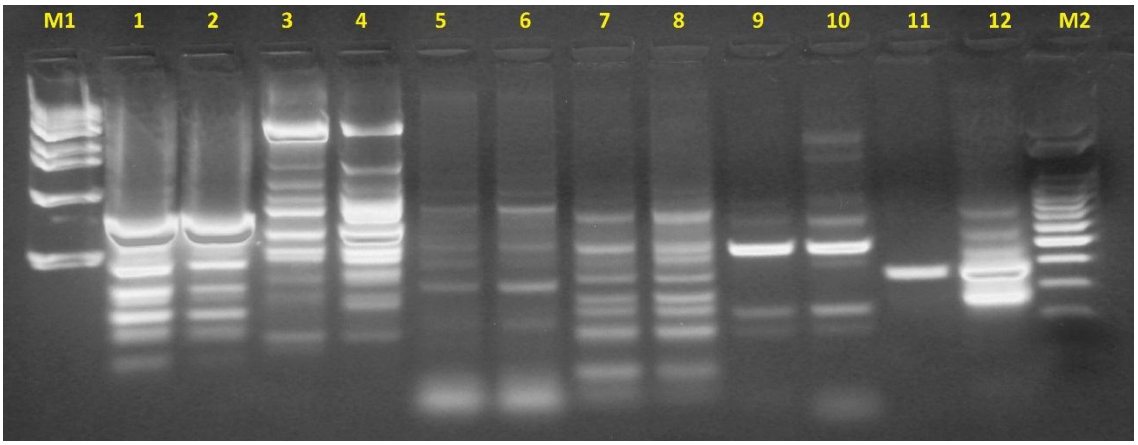


Plate 3. Primer test: PCR amplification products by 6 different decamer random primers using DNA from different brinjal varieties

(Lane 1-2: OPA-18; Lane 3-4: OPB-04; Lane 5-6: OPB-06; Lane 7-8: OPB-08; Lane 9-10: OPF-08; Lane 11-12: OPW-08. M1 = 1Kb DNA ladder and M2= 100 bp DNA ladder)

4.3 RAPD banding pattern and DNA polymorphism in Brinjal

The selected five primers produced comparatively maximum number of high intensity band with minimal smearing, good technical resolution and sufficient variation among different cultivars. These five primers (OPB-04, OPB-08, OPD-02, OPP-13 and OPW-08) generated total 44 distinct and differential amplification bands i.e. average 8.8 bands per primer. Out of them 5.6 polymorphic bands per primer on an average 4 different DNA band were amplified in each brinjal cultivars. The size of the bands ranges from 198 to 2898 bp. The highest number of bands was generated by primer OPD-02 (Table 5). Besides, the primer OPB-04, OPB-08, OPP-13 and OPW-08 also generated considerable number of polymorphic bands.

Percentage of polymorphic loci in the present study ranged from 37.5% to 87.5%. The Maximum percentage of polymorphic loci (87.5%) was detected by primer OPW-08 while primer OPB-04 detected the least percentage (37.5%). The level of polymorphism (63.64%) indicated the effectiveness of RAPD technique to study substantial amount of polymorphisms or diversity among the different genotypes of brinjal.

The banding pattern of 11 brinjal genotypes using primer OPB-04, OPB-08, OPD-02, OPP-13 and OPW-08 are shown in plate 4, 5, 6, 7 and 8 respectively. No. of RAPD markers scored for each individual of 11 varieties for each primer are presented in Table 5.

Table 5. RAPD primers with corresponding band score and their size range together with number and percentage of polymorphic loci observed in 11 brinjal varieties

Name of RAPD primer	Sequence of the primer	GC content (%)	No. of bands scored	Size ranges (bp) observed	No. of polymorphic bands	Percentage of polymorphic loci
OPB-04	GGACTGGAGT	60%	8	206-2836	3	37.5
OPB-08	GTCCACACGG	70%	8	198-1157	5	62.5
OPD-02	GGACCCAACC	70%	12	419-3898	9	75
OPP-13	GGAGTGCCTC	70%	8	222-870	4	50
OPW-08	GACTGCCTCT	60%	8	267-897	7	87.5
Total	**-**	**330**	**44**	**-**	**28**	**63.64**

Several scientists reported similar type of observations on brinjal with RAPD primers. The polymorphic amplification bands ranged from 3-9 on an average 5.6 bands per primer. Verma *et al.* (2012) analyzed 29 popular Indian brinjal varieties with 11 RAPD markers and obtained 5.58 bands per primer. Thakkar *et al.* (2014) analyzed 10 brinjal genotypes with 10 RAPD primers, which resulted 5.7 bands per primer. Jin *et al.* (2007) analysed the genetic diversity among 53 aubergine germplasms by 9 random amplified polymorphic DNA (RAPD) markers and obtained 5.11 bands per primer. Islam *et al.* (2014) found 5.67 bands per primer while studying 15 brinjal germplasm with three decamer RAPD primers. Thus, the results from previous studies are very similar with the present study.

In this study, the percentage of polymorphic loci was 63.64%, which is also justified by several previous experiments. Sharmin *et al.* (2011) observed 57.58% polymorphism in their study on eight eggplant cultivars using 3 RAPD decamer primers. Jin *et al.* (2007) found 56.79% polymorphism among 53 aubergine germplasms with 9 RAPD markers. Biswas *et al*. (2009) detected 57.89% polymorphic loci using four RAPD markers among ten eggplant varieties. These results are very close to the current findings. Although some scientists also observed higher level of polymorphism using RAPD markers on brinjal. Thakkar *et al.* (2014) analyzed 10 brinjal genotypes using 10 RAPD primers and observed 83.43% average polymorphism. Koundal *et al.* (2006) characterized 38 brinjal accessions with 30 RAPD primers and obtained 72.4% polymorphism. Islam *et al.* (2014) found 70.59% polymorphism in their investigation of 15 brinjal germplasm with 3 decamer RAPD primers. These results show higher level of polymorphism than the present study.

Relatively lower level of polymorphism is also found in various experiments. Demir *et al.* (2010) found only 29% polymorphism with 11 decamer RAPD primers. Tiwari *et al*. (2009) carried out an experiment on 19 advanced cultivars and landraces of brinjal using 29 RAPD primers and observed 27.5% polymorphic loci.

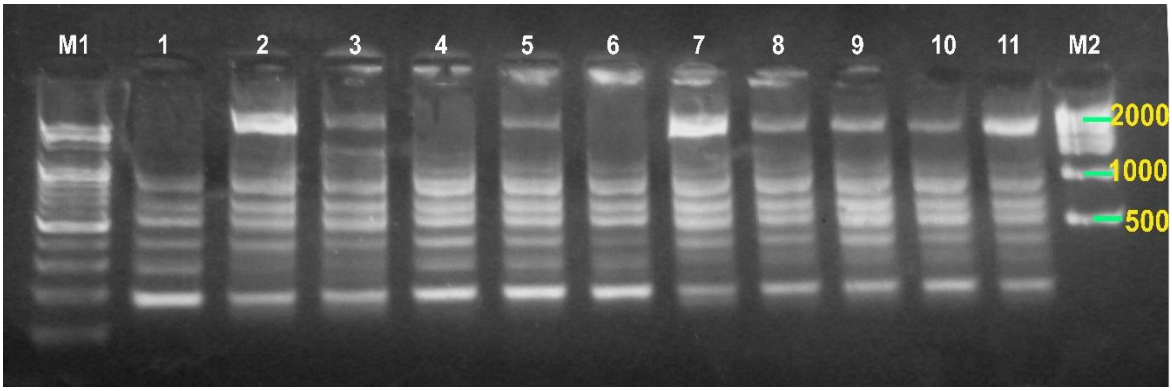


Plate 4. RAPD profile of 11 brinjal varieties using primer **OPB-04.**

(Lane 1: BARI begun 1, Lane 2: BARI begun 4, Lane 3: BARI begun 5, Lane 4: BARI begun 6, Lane 5: BARI begun 7, Lane 6: BARI begun 8, Lane 7: BARI begun 9, Lane 8: BARI begun 10, Lane 9: BARI hybrid begun 1, Lane 10: BARI hybrid begun 3, Lane 9: BARI hybrid begun 4. Ms: M1=100 bp DNA ladder and M2: 1 kb DNA ladder)

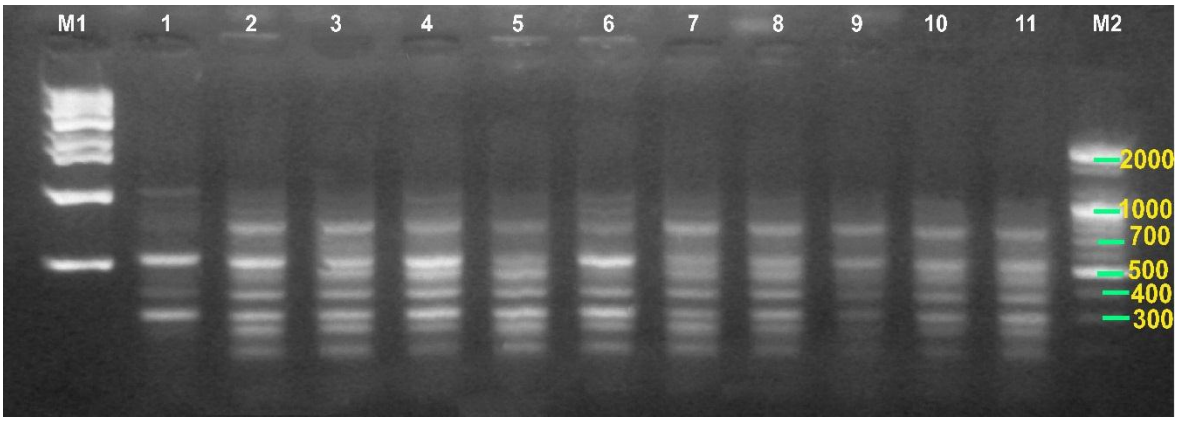


Plate 5. RAPD profile of 11 brinjal varieties using primer **OPB-08**

(Lane 1: BARI begun 1, Lane 2: BARI begun 4, Lane 3: BARI begun 5, Lane 4: BARI begun 6, Lane 5: BARI begun 7, Lane 6: BARI begun 8, Lane 7: BARI begun 9, Lane 8: BARI begun 10, Lane 9: BARI hybrid begun 1, Lane 10: BARI hybrid begun 3, Lane 9: BARI hybrid begun 4. Ms: M1=1Kb DNA ladder and M2: 100bp DNA ladder)

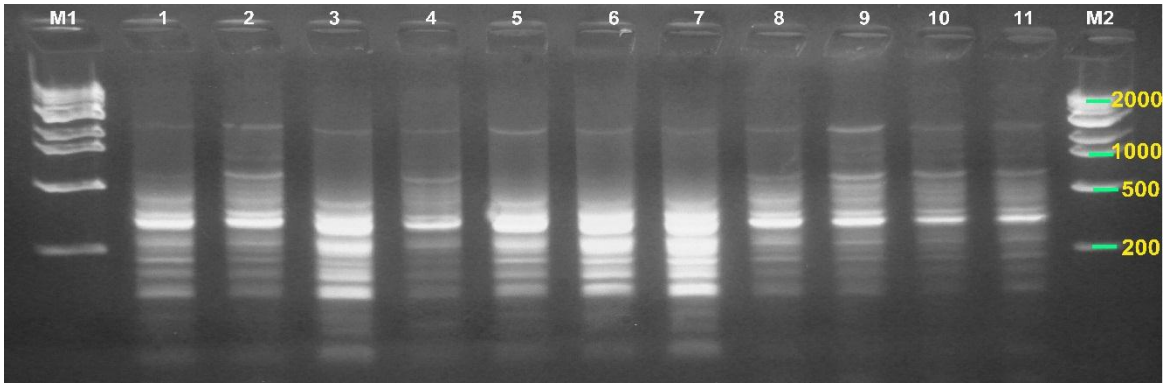


Plate 6. RAPD profile of 11 brinjal varieties using primer **OPD-02**

(Lane 1: BARI begun 1, Lane 2: BARI begun 4, Lane 3: BARI begun 5, Lane 4: BARI begun 6, Lane 5: BARI begun 7, Lane 6: BARI begun 8, Lane 7: BARI begun 9, Lane 8: BARI begun 10, Lane 9: BARI hybrid begun 1, Lane 10: BARI hybrid begun 3, Lane 9: BARI hybrid begun 4. Ms: M1=1Kb DNA ladder and M2: 1kb DNA ladder)

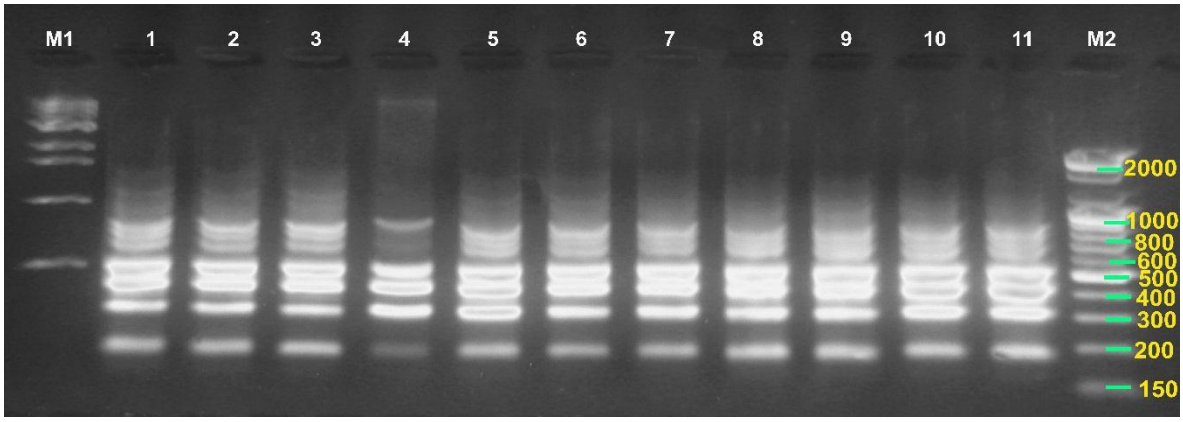


Plate 7. RAPD profile of 11 brinjal varieties using primer **OPP-13**

(Lane 1: BARI begun 1, Lane 2: BARI begun 4, Lane 3: BARI begun 5, Lane 4: BARI begun 6, Lane 5: BARI begun 7, Lane 6: BARI begun 8, Lane 7: BARI begun 9, Lane 8:BARI begun 10, Lane 9: BARI hybrid begun 1, Lane 10: BARI hybrid begun 3, Lane 9: BARI hybrid begun 4. Ms: M1=1Kb DNA ladder and M2: 100bp DNA ladder)

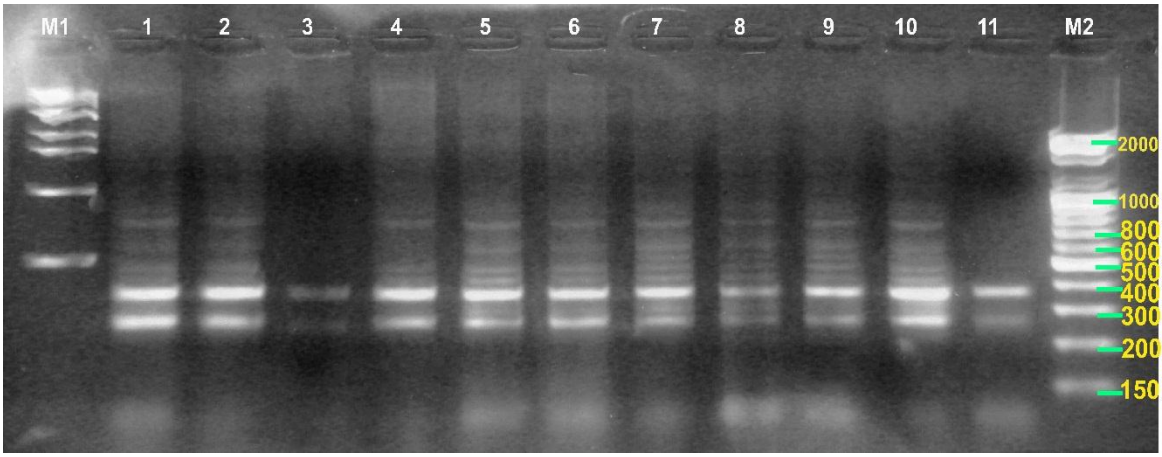


Plate 8: RAPD profile of 11 brinjal varieties using primer **OPW-08**

(Lane 1: BARI begun 1, Lane 2: BARI begun 4, Lane 3: BARI begun 5, Lane 4: BARI begun 6, Lane 5: BARI begun 7, Lane 6: BARI begun 8, Lane 7: BARI begun 9, Lane 8: BARI begun 10, Lane 9: BARIhybrid begun 1, Lane 10: BARI hybrid begun 3, Lane 9: BARI hybrid begun 4. Ms: M1=1Kb DNA ladder and M2: 100bp DNA ladder)

4.4 Nei's (1972) Gene Diversity and Gene Frequency

DNA polymorphisms were detected according to band presence and absence. Absence of bands may be caused by failure of primers to anneal a site in some individuals due to nucleotide sequence differences or by insertions or deletions between primer sites (Clark and Lanigan, 1993).

The five primers used in the present study showed different levels of gene diversity and gene frequency. Nei's (1972) Gene diversity ranged from 0 to 0.4959 and gene frequency ranged from 0.0909 to 1.00. The highest gene frequency (1.00) as well as the lowest gene diversity (0) was shown by the primer OPB-04 (at 817, 650, 516, 415 and 216 bp), OPB-08 (at 555, 387 and 304 bp), OPD-02 (at 2898,718 and 665 bp), OPP-13 (at 502, 412, 331 and 222 bp) and OPW-08 (at 360 bp)

The highest gene diversity was shown by the primer OPB-04 (at 1127 bp), OPD-02 (at 1101, 518 and 476 bp) and OPW-08 (at 633, 563 and 469 bp). On the other hand the lowest gene frequency was observed at 1157 bp by OPB-08, at 1448 bp by OPD-02 and at 870 bp by OPP-13.

Table 6. Molecular sizes of the loci, their diversities and frequencies among 11 brinjal varieties

RAPD Primers	Locus No.	Locus Size (bp)	Nei's (1972) Gene Diversity	Gene Frequency
OPB-04	1	2836	0.3967	0.7273
	2	1127	**0.4959**	0.5455
	3	817	0.0000	**1.0000**
	4	650	0.0000	**1.0000**
	5	516	0.0000	**1.0000**
	6	415	0.0000	**1.0000**
	7	294	0.4628	0.3636
	8	206	0.0000	**1.0000**
OPB-08	1	1157	0.1653	**0.0909**
	2	793	0.1653	0.9091
	3	555	0.0000	**1.0000**
	4	485	0.3967	0.7273
	5	387	0.0000	**1.0000**
	6	304	0.0000	**1.0000**
	7	248	0.3967	0.7273
	8	198	0.4628	0.6364
OPD-02	1	2898	0.0000	**1.0000**
	2	1448	0.1653	**0.0909**
	3	1101	**0.4959**	0.5455
	4	979	0.1653	0.9091
	5	828	0.1653	0.9091

Table 6 (cont'd).

	6	718	0.0000	**1.0000**
	7	665	0.0000	**1.0000**
	8	569	0.1653	0.9091
	9	518	0.4959	0.5455
	10	476	0.4959	0.5455
	11	445	0.4628	0.3636
	12	419	0.2975	0.8182
OPP-13	1	870	0.1653	**0.0909**
	2	795	0.1653	0.9091
	3	710	0.1653	0.9091
	4	631	0.1653	0.9091
	5	502	0.0000	**1.0000**
	6	412	0.0000	**1.0000**
	7	331	0.0000	**1.0000**
	8	222	0.0000	**1.0000**
OPW-08	1	897	0.4628	0.3636
	2	760	0.3967	0.7273
	3	633	**0.4959**	0.4545
	4	563	**0.4959**	0.5455
	5	469	**0.4959**	0.5455
	6	410	0.4628	0.3636
	7	360	0.0000	**1.0000**
	8	267	0.2975	0.8182

The overall gene diversity was obtained 0.2186 ranges from 0.00 to 0.4959 in this study which is very close to the previous findings of several scientists. Biswas *et al*. (2009) found 0.23 gene diversity among 10 promising brinjal varieties with RAPD markers. Sing *et al*. (2005) observed high level of gene diversity in eggplant.

4.5 Inter-variety similarity indices (S_{ij})

The similarity indices (S_{ij}) for different accession pairs with five different primers and their average are shown in Table 7.

In the present study, inter-varietal means of the pair wise similarity indices (S_{ij}) ranged from 67.03% to 97.61%. The highest similarity indices of 97.61% was found between BARI begun 7 and BARI begun 9 varietal pair. So, genetic distance was lower between that pair than rest of the varietal pairs. On the other hand, BARI begun 6 and BARI begun 10 pair showed least Inter-variety similarity indices of 67.03% and genetic distance was higher between that pair than rest of the varietal pairs.

All the 55 varietal pairs were not homogenous at different number of loci and with different primers. Therefore, this study clearly indicates that there was a high level of genetic variation among different varieties.

Table 7. RAPD band sharing percentage or inter-variety similarity indices among the brinjal varieties across five primers

Varietal pairs	Band sharing values (%)					
	OPB 04	OPB 08	OPD 02	OPP 13	OPW 08	Average
BARI begun 1 vs. BARI begun 4	83.33	54.54	80	100	100	83.57
BARI begun 1 vs. BARI begun 5	83.33	54.54	88.89	100	50	75.35
BARI begun 1 vs. BARI begun 6	92.31	54.54	57.14	66.67	75	69.13
BARI begun 1 vs. BARI begun 7	85.71	54.54	88.89	100	54.54	76.74
BARI begun 1 vs. BARI begun 8	92.31	60	94.73	100	54.54	80.32
BARI begun 1 vs. BARI begun 9	76.92	54.54	94.73	100	54.54	76.15
BARI begun 1 vs. BARI begun 10	76.92	60	88.89	100	50	75.16
BARI begun 1 vs. BARI hybrid begun 1	76.92	75	88.89	100	60	80.16
BARI begun 1 vs. BARI hybrid begun 3	83.33	60	75	100	60	75.67
BARI begun 1 vs. BARI hybrid begun 4	83.33	60	75	100	80	79.67
BARI begun 4 vs. BARI begun 5	100	100	80	100	50	86.00
BARI begun 4 vs. BARI begun 6	76.92	100	62.50	66.67	75	76.22
BARI begun 4 vs. BARI begun 7	85.7 1	100	80	100	54.54	84.05
BARI begun 4 vs. BARI begun 8	76.92	92.31	85.71	100	54.54	81.90
BARI begun 4 vs. BARI begun 9	92.31	100	85.71	100	54.54	86.51
BARI begun 4 vs. BARI begun 10	92.31	92.31	90	100	50	84.92
BARI begun 4 vs. BARI hybrid begun 1	92.31	72.72	90	100	60	83.00
BARI begun 4 vs. BARI hybrid begun 3	100	92.31	77.78	100	60	86.02
BARI begun 4 vs. BARI hybrid begun 4	100	92.31	77.78	100	80	90.02

Table 7 (cont'd).

BARI begun 5 vs. BARI begun 6	76.92	100	57.14	66.67	33.33	66.81
BARI begun 5 vs. BARI begun 7	85.71	100	100	100	22.22	81.59
BARI begun 5 vs. BARI begun 8	76.92	92.31	94.73	100	22.22	77.24
BARI begun 5 vs. BARI begun 9	92.31	100	94.73	100	22.22	81.85
BARI begun 5 vs. BARI begun 10	92.31	92.31	77.78	100	100	92.48
BARI begun 5 vs. BARI hybrid begun 1	92.31	72.72	77.78	100	25	73.56
BARI begun 5 vs. BARI hybrid begun 3	100	92.31	75	100	25	78.46
BARI begun 5 vs. BARI hybrid begun 4	100	92.31	75	100	66.67	86.80
BARI begun 6 vs. BARI begun 7	93.33	100	57.14	66.67	76.92	78.81
BARI begun 6 vs. BARI begun 8	100	92.31	53.33	66.67	76.92	77.85
BARI begun 6 vs. BARI begun 9	85.71	100	53.33	66.67	76.92	76.53
BARI begun 6 vs. BARI begun 10	85.71	92.31	57.14	66.67	33.33	67.03
BARI begun 6 vs. BARI hybrid begun 1	85.71	72.72	71.42	66.67	83.33	75.97
BARI begun 6 vs. BARI hybrid begun 3	76.92	92.31	66.67	66.67	83.33	77.18
BARI begun 6 vs. BARI hybrid begun 4	76.92	92.31	66.67	66.67	40	68.51
BARI begun 7 vs. BARI begun 8	93.33	92.31	94.73	100	100	96.07
BARI begun 7 vs. BARI begun 9	93.33	100	94.73	100	100	97.61
BARI begun 7 vs. BARI begun 10	93.33	92.31	77.78	100	22.22	77.13
BARI begun 7 vs. BARI hybrid begun 1	93.33	72.72	77.78	100	93.33	87.43
BARI begun 7 vs. BARI hybrid begun 3	85.71	92.31	75	100	93.33	89.27

Table 7 (cont'd).

BARI begun 7 vs. BARI hybrid begun 4	85.71	92.31	75	100	40	78.60
BARI begun 8 vs. BARI begun 9	85.71	92.31	100	100	100	95.60
BARI begun 8 vs. BARI begun 10	85.71	83.33	84.21	100	22.22	75.09
BARI begun 8 vs. BARI hybrid begun 1	85.71	80	84.21	100	93.33	88.65
BARI begun 8 vs. BARI hybrid begun 3	76.92	83.33	70.59	100	93.33	84.83
BARI begun 8 vs. BARI hybrid begun 4	76.92	83.33	70.59	100	40	74.17
BARI begun 9 vs. BARI begun 10	100	92.31	84.21	100	22.22	79.75
BARI begun 9 vs. BARI hybrid begun 1	100	72.72	84.21	100	93.33	90.05
BARI begun 9 vs. BARI hybrid begun 3	92.31	76.92	70.59	100	93.33	86.63
BARI begun 9 vs. BARI hybrid begun 4	92.31	92.31	70.59	100	40	79.04
BARI begun 10 vs. BARI hybrid begun 1	100	80	100	100	25	81.00
BARI begun 10 vs. BARI hybrid begun 3	92.31	83.33	87.50	100	25	76.63
BARI begun 10 vs. BARI hybrid begun 4	92.31	83.33	87.50	100	66.67	85.96
BARI hybrid begun 1 vs. BARI hybrid begun 3	92.31	80	87.50	100	85.71	89.10
BARI hybrid begun 1 vs. BARI hybrid begun 4	92.31	80	87.50	100	44.44	80.85
BARI hybrid begun 3 vs. BARI hybrid begun 4	100	100	100	100	44.44	88.89

Many scientists reported similar type of observations on different brinjal varieties with RAPD primers. Sing *et al*. (2005) observed the level of polymorphism among 28 eggplant accessions was 0.05 to 0.82, indicating a wide and diverse base. Karihaloo and Gottlieb (1995) reported similarity of 52 accessions of *Solanum* and found highest similarity (0.947) between *S. insanum* and *S. melongena*. Pair-wise variety comparison of the varieties showed that inter-cultivar similarity indices for Kazla vs. Nayantara (92.54%), for Singnath vs. BARI Begun-08 (91.07%) were higher than all other cultivar pairs. On the other hand, inter-cultivar similarity index for Khatkhatia vs. BARI Eggplant Line-083 (41.67%) cultivar was lower than all the other cultivar pairs. Among 10 Jordanian eggplant landraces E22 and E23 showed highest distant similarity 80.5% while the most distant genotypes were E12 and Ec5 having lowest similarity 4.8% (Sadder *et al*. 2004).

4.6 Nei's (1972) genetic identity and genetic distance

Pair-wise comparisons of Nei's (1972) genetic identity between 11 brinjal cultivars were calculated from the combined data of the five primers and the value ranges from 0.5909 to 0.9545.The highest Nei's genetic identity (0.9545) was observed in BARI begun 9 vs. BARI begun 7 varietal pair whereas the lowest genetic identity (0.5909) was estimated in BARI begun 6 vs. BARI begun 1 varietal pair (Table 8). The differences between highest and lowest genetic identity indicates the presence of variability among 11 accessions of brinjal.

Nei's (1972) genetic distance between 11 brinjal cultivars ranges from 0.0465 to 0.5261. The highest Nei's genetic distance (0.5261) was observed in BARI begun 6 vs. BARI begun 1 varietal pair whereas the lowest genetic distance (0.0465) was estimated in BARI begun 9 vs. BARI begun 7 varietal pair (Table 8).

Table 8: Summary of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) values between 11 brinjal varieties

Acc. No.	BARI begun 1	BARI begun 4	BARI begun 5	BARI begun 6	BARI begun7	BARI begun 8	BARI begun 9	BARI begun 10	BARI hybrid begun 1	BARI hybrid begun 3	BARI hybrid begun 4
BARI begun 1	****	0.7955	0.7500	**0.5909**	0.6818	0.7500	0.6818	0.7500	0.7500	0.6818	0.7500
BARI begun 4	0.2288	****	0.8636	0.6591	0.7500	0.7273	0.7955	0.8636	0.7727	0.7955	0.8636
BARI begun 5	0.2877	0.1466	****	0.6136	0.7955	0.7273	0.7955	0.8636	0.6818	0.7500	0.8636
BARI begun 6	**0.5261**	0.4169	0.4884	****	0.6818	0.6591	0.6364	0.6591	0.6591	0.6818	0.6591
BARI begun 7	0.3830	0.2877	0.2288	0.3830	****	0.9318	**0.9545**	0.7045	0.7955	0.8182	0.7045
BARI begun 8	0.2877	0.3185	0.3185	0.4169	0.0706	****	0.9318	0.6818	0.8182	0.7500	0.6364
BARI begun 9	0.3830	0.2288	0.2288	0.4520	**0.0465**	0.0706	****	0.7500	0.8409	0.8182	0.7045
BARI begun 10	0.2877	0.1466	0.1466	0.4169	0.3502	0.3830	0.2877	****	0.8182	0.7500	0.8636
BARI hybrid begun 1	0.2877	0.2578	0.3830	0.4169	0.2288	0.2007	0.1733	0.2007	****	0.8409	0.7727
BARI hybrid begun 3	0.3830	0.2288	0.2877	0.3830	0.2007	0.2877	0.2007	0.2877	0.1733	****	0.8864
BARI hybrid begun 4	0.2877	0.1466	0.1466	0.4169	0.3502	0.4520	0.3502	0.1466	0.2578	0.1206	****

Whereas, several scientists reported similar type of observations in brinjal genotypes. Verma *et al.* (2012) studied 29 popular Indian brinjal varieties and found a range of 0.07 to 0.78 genetic distance with an average of 0.33 based on RAPD markers among all the varieties.

Biswas *et al*. (2009) studied genetic relationship among 10 eggplant varieties using RAPD markers. The experiment showed highest genetic distance value 0.48 and lowest genetic distance 0.14. Islam *et al.* (2014) observed a range of 0.8873 to 0.1525 genetic distance and lowest genetic identity was found 0.4118 among 15 brinjal germplasm with three random amplified polymorphic DNA (RAPD) marker.

4.7 UPGMA Dendrogram

Dendrogram based on Nei's (1972) genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated the segregation of 11 genotypes of brinjal into two main clusters: A and B. The first major cluster 'A' had only one genotype (BARI begun 6) and the second major cluster 'B' had rest of ten genotypes. The second major cluster was subdivided into two minor clusters (C & D) in which one cluster (D) had only one genotype (BARI begun 1) and the other cluster (C) had rest of the nine. This minor cluster was also subdivided into two clusters (E & F). In which cluster (E) is divided into two sub clusters (G and H). BARI hybrid begun 1 formed cluster (G) and BARI begun 7, BARI begun 8 and BARI begun 9 were grouped in cluster (H). Cluster (I) and (J) were the subdivision of cluster (F). BARI hybrid begun 3 and BARI hybrid begun 4 formed cluster (I) and BARI begun 4, BARI begun 5 and BARI begun 10 were grouped in cluster (J).

The result indicates that the low or high level genetic distance exists between varieties with their same or different origins. BARI begun 6 vs. BARI begun 1 showed highest Nei's genetic distance (0.5261) as they are released from different parental origin. On the other hand BARI began 9 vs. BARI begun 8

varietal pair showed lowest genetic distance (0.0706) as they are released from same parental origin. This variation can be created by geographical origin. The result also reveals that the genetic base among these brinjal varieties is rather narrow. Collection of diverse germplasm from centers of diversity may borden the genetic base. RAPD markers provide a fast, efficient technique for variability assessment that complements methods currently being used in genetic resource management.

The above results are shown in Figure 1. The results indicate that, different level of genetic identity and distance present between the studied 11 brinjal genotypes.

Cluster analysis on brinjal genotypes was also performed by several scientists. In the study conducted by Biswas *et al*. (2009) the UPGMA dendrogram based on genetic distance segregated the 10 varieties of eggplant into two main clusters. Dohazari, Kazla, Nayantara and ISD-006 were grouped together in cluster I whereas Uttara, Islampuri, Khatkhatia, Singnath, BARI Begun-08 and Eggplant Line-083 into cluster II. The clusters also divided into sub-clusters and sub-clusters further into groups.

Islam *et al.* (2014) constructed an Unweighted Pair-Group Method of Arithmetic Means (UPGMA) dendrogram from genetic distance and all the 15 brinjal cultivars were grouped into five clusters. The dendrogram (UPGMA) constructed by Sharmin *et al.* (2011) from Nei's genetic distance produced 2 main clusters of the parents and F5 offsprings.

Eggplant germplasms of the Indian subcontinent are very diverse. Wide variation in the desirable genotypes in different regions substantiates the high level of genetic variability observed. High degree of diversity of species belonging to *Solanum* may be attributable to the fact that it is an ancient plant (Whalen, 1979).

RAPD and other discontinuous markers can serve as means of genetic distances to establish phylogenetic relationships among taxa (Karihaloo and Gottieb, 1995; Rodriguez *et al.,* 1999; Rabey *et al.,* 2002). Estimation of genetic differences and discrimination of genetic relationship between *Solanum spp.* are for utilization of plant genetic resources.

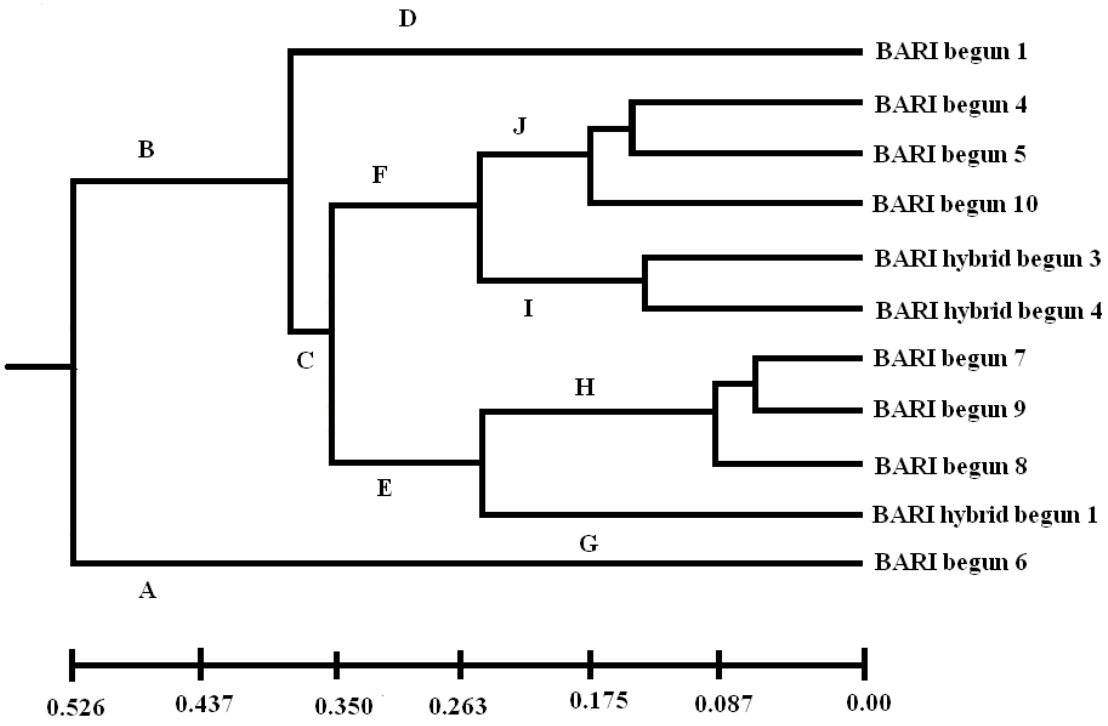
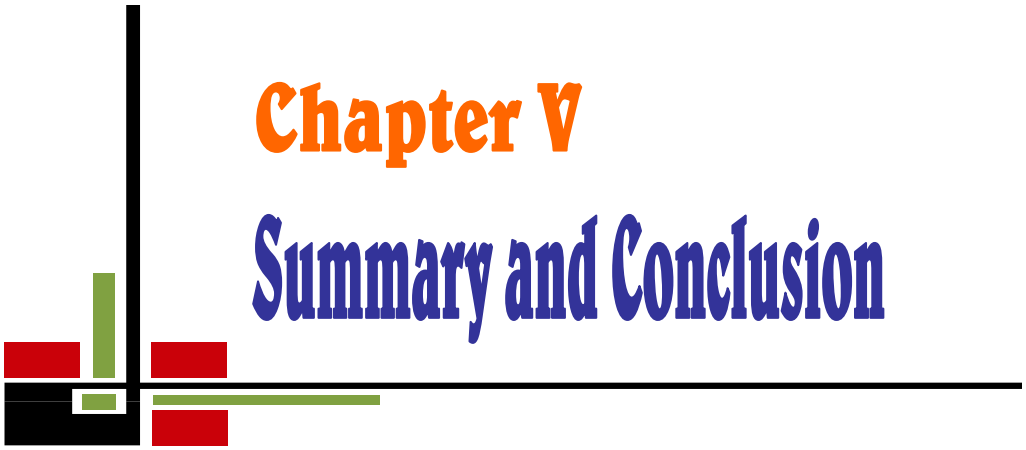


Figure 1. Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's (1972) genetic distance, summarizing data on differentiation for 11 brinjal varieties according to RAPD analysis



Chapter V

Summary and Conclusion

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SUMMARY AND CONCLUSION

Eggplant or brinjal (*Solanum melongena* L.) is an important and popular vegetable crop cultivated in the tropics and subtropics. Its position is second in vegetable crops in terms of production next to potato in Bangladesh. A large number of eggplant cultivars are grown in Bangladesh, which show a wide range of variations in yield performance and disease reaction. Eggplants present a high morphogenetic potential that is useful for the developmental studies as well as for establishing biotechnological approaches to produce improved varieties. The genetic characterization of these varieties is yet to be done using molecular markers. The aim of the present research work was to molecular characterization and to reveal DNA level variation of the 11 brinjal varieties using RAPD analysis. The present experiment was conducted at Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207. In this study, the genetic variation of 11 brinjal varieties (BARI begun 1, BARI begun 4, BARI begun 5, BARI begun 6, BARI begun 7, BARI begun 8, BARI begun 9, BARI begun 10, BARI hybrid begun 1, BARI hybrid begun 3 and BARI hybrid begun 4) were analyzed and amplified with RAPD markers by Polymerase Chain Reaction (PCR) using five primers (OPA-18, OPB-04, OPB-08, OPD-02, OPP-13 and OPW-08).

These primers generated total 44 distinct and differential amplification bands with an average of 5.6 polymorphic bands per primer. The highest percentage of polymorphic bands (87.5%) was generated by primer OPW-08 while primer OPB-04 generated the least percentage of polymorphic bands (37.5%). The overall gene diversity was ranged from 0.2186 to 0.4959 in this study.

Inter-varietal means of the pair wise similarity indices (S_{ij}) ranged from 67.03 to 97.61. The highest similarity indices of 97.61% were found between BARI

begun 7 and BARI begun 9. Genetic distance was lower between that pair than rest of the varietal pairs. BARI begun 6 and BARI begun 10 showed least inter-variety similarity indices 67.03% and genetic distance was higher between that pair than rest of the varietal pairs. The highest Nei's genetic identity (0.9545) was detected in BARI begun 9 vs. BARI begun 7 varietal pair whereas the lowest genetic identity (0.6136) was estimated in BARI begun 6 vs. BARI begun 5 varietal pair. The highest Nei's genetic distance (0.5261) was observed in BARI begun 6 vs. BARI begun 1 varietal pair whereas the lowest genetic distance (0.0706) was estimated in BARI begun 9 vs. BARI begun 8 varietal pair.

The UPGMA Dendrogram based on Nei's (1972) genetic distance between different pairs was correlated with their sources of origin. The dendrogram indicated the segregation of 11 genotypes of brinjal into two main clusters. The first major cluster had only one genotype (BARI begun 6) and the second major cluster had rest of ten genotypes. The second major cluster was divided into two minor clusters in which one cluster had only one genotype (BARI begun 1) and the other cluster had rest of the nine.

Though, larger number of samples and higher number of primers would be necessary to generate and construct an appropriate genetic relationship, sample identification and analysis of genetic variation among different varieties and cultivars is widely acceptable by all concern. Using larger number of samples and higher number of primers could be useful in future study.

The present work was the preliminary study to assess genetic variation of brinjal varieties and it had some limitations in term of limited number of individuals and varieties 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 sustaining the genetic qualities of brinjal in Bangladesh:

1. Large number of varieties and higher number of primers should be studied with adequate replications to increase accuracy.
2. Details survey work should be conducted using more molecular markers for obtaining diagnostic loci for brinjal varieties.
3. Other molecular markers such as SNP, AFLP, micro-satellite, etc. should be developed for brinjal varieties of Bangladesh.

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