PHENOTYPIC CHARACTERIZATION AND GENETIC DIVERSITY ANALYSIS OF EGGPLANT (*Solanum spp.*) GERMPLASM USING SSR MARKER

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CERTIFICATE

This is to certify that the thesis entitled ***"PHENOTYPIC CHARACTERIZATION AND GENETIC DIVERSITY ANALYSIS OF EGGPLANT (Solanum spp.) GERMPLASM USING SSR MARKER"*** *submitted to the Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (MS) in BIOTECHNOLOGY****, embodies the result of a piece of bonafide research work carried out by* ***KASHPIA TASRIN,*** *Registration No.* ***10-03823*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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ABSTRACT

An experiment was carried out with twenty five local and two wild relatives (*Solanum sisymbriifolium* and *S. torvum*) of eggplant at the research field and in the Biotechnology Laboratory of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, during October 2015 to June 2016 to investigate the phenotypic characterization and molecular diversity analysis of different local eggplant germplasm and its wild relatives. The morphological study revealed diversified characters among all the genotypes. Variations were observed in color of leaf vein, stem as well as fruit size and shape. Maximum yield per plant was recorded in Salta Begun and it was 9.8 kg. The minimum yield per plant was observed in both the wild species (3.8 kg) as they produced very small fruits. Correlation of yield contributing traits separated all the genotypes into four clusters (A, B, C, and D). Among them cluster D had five genotypes together with the wild species indicated their relatedness. Five well-known SSR primers were used for the molecular characterization of the genotypes. Ten alleles, ranged from 1 to 3 alleles per locus and an average of 2.0 were detected. Among five primers three were able to produce polymorphic bands and the total number of polymorphic bands was five. The highest (2) number of bands was observed in SSR primers EPSSR82 and smSSR01. The Polymorphism Information Content (PIC) of SSR markers ranged from 0.37 (smSSR01) to 0.67 (EPSSRR82) with an average value of PIC = 0.54. Gene diversity ranges from 0.49 (smSSR01) to 0.72 (EPSSR82), with an average value of 0.61. The value of pair-wise comparisons of Nei's (1972) genetic distance (D) and identity between varieties was computed from combined data for the five primers, ranged from 0.20 to 1.00, with an average value of 0.60. Multivariate cluster analysis on the basis of Nei's genetic distance and identity revealed that within those 27 genotypes there surely do exist considerable diversity. UPGMA method separated the segregation of 27 genotypes into two major clusters (I and II). From the clusters, wild species *Solanum torvum* belonged to the sub-cluster (IIb), that revealed its distinct variation from the others. On the other hand, wild species *Solanum sisymbriifolium* showed a close relatedness by forming the same cluster together with thirteen local eggplant genotypes. This experiment brought a great opportunity for eggplant improvement as the local genotypes had a close relation with the wild species.

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ABBREVIATIONS AND ACRONYMS

FULL WORD	ABBREVIATION
Amplified Fragment Length Polymorphism	AFLP
And others (at elli)	*et al.*
Bangladesh Agricultural Research Institute	(BARI)
Bangladesh Bureau of Statistics	BBS
Base pair	bp
Centimeter	cm
Cetyl Trimethyl Ammonium Bromide	CTAB
Continued	Cont'd
Degree celsius	^{o}C
Deoxyribonucleic acid	DNA
Deionized water	dH_2O
Ethylene Diamine Tetra Acetic Acid	EDTA
Etcetera	etc.
Ethidium Bromide	Et-Br
Food and Agricultural Organization Statistical Databases	FAOSTAT
Gram	g
Gram per Liter	g/L
Inter Simple Sequence Repeats	ISSRs
Micro liter	µl
Micro mole	µM
Mili liter	ml
Namely	*viz.*
Negative logarithm of hydrogen ion concentration (-log[H+])	pH
Percent	%

ABBREVIATIONS AND ACRONYMS (Cont'd)

FULL WORD	ABBREVIATION
Polymerase Chain Reaction	PCR
Random Amplified Polymorphic DNA	RAPD
Randomized Complete Block Design	RCBD
Restriction Fragment Length Polymorphism	RFLP
Ribonucleic acid	RNA
Rotation per minute	rpm
Single Nucleotide Polymorphism	SNP
Simple Sequence Repeat	SSR
Sodium chloride	NaCl
Sodium Dodecyl Sulphate	SDS
Species	spp.
Solanum	*S.*
Thermophilus aquaticus	Taq
Tris Boric Acid EDTA	TBE
Tris-EDTA	TE
tons	t
Unweighted Pair Group of Arithmetic mean	UPGMA
Ultra Violet	UV
Volt	V

Chapter One

INTRODUCTION

Eggplant (*Solanum spp.* 2n = 2x = 24) belongs to the plant family of Solanaceae. The term eggplant is used in Australia and North America while British English uses the term aubergine. Brinjal is the common name of this plant in South Africa, South Asia, and Southeast Asia. Just like other Solanaceous crops, they grow in the same manner as tomatoes, pepper hanging from the vines of a plant that grows several feet in height. Eggplant has served as a model member of this family largely because of it's enriched cytogenetic, genetic, as well as physical maps (Fukuoka *et al*., 2010). Eggplant is placed in *Solanum* genus and includes wide genotypic and phenotypic variation. Eggplant is believed to have originated in Asia, in the Indo-Burmese region (Vavilov, 1926; Ishiiki *et al*., 1994) but the most recent DNA sequencing studies suggested that eggplant arose from Africa (Weese, 2007). It has been well known since BCIII and cultivated for 1,500 years in Asia (Kashyap *et al.*, 2003). It is cultivated as a perennial in tropical areas, while it is cultivated as annual in subtropical areas (Kowalska, 2008). But the wild relatives of eggplant are known to originate from Africa.

At present eggplant is the sixth most important vegetable after tomato, watermelon, onion, cabbage, and cucumber and the most important *Solanum* crop native to the Old World (FAO, 2016). At the global level, it has been one of the crops with the greatest increase in production in the last years, with total production rising by 59% in a decade, from $31.0 \cdot 10^6$ t in 2004 to $49.3 \cdot 10^6$ t in 2013 (FAO, 2016).

Eggplants have a remarkable demand and are considered as the second important vegetable after potato in Bangladesh (DAE, 2016). Its availability, year round cultivation pattern and taste make it more desirable vegetable in Bangladesh. As eggplant is a native plant of Indian sub-continent which surely can define its abundance in this region. Though it is cultivated almost all over the country its production is not as good as expected for being an ancient plant of this region. In the year 2014-15, total area devoted to eggplant cultivation was 1,22,014 acres with an

annual production of 4,50,146 metric tons together with Kharif season (1,39,792 metric tons) and Rabi season (3,10,354 metric tons) (BBS, Yearbook,2015).

Eggplant has a number of health benefits, they do contain an impressive array across the board of many vitamins and minerals, such as excellent amounts of fiber, folate, potassium, and manganese, as well as vitamins C, K, and B6, phosphorus, copper, thiamin, niacin, magnesium, and pantothenic acid. Phenolic compounds in eggplant contain significant amounts of chlorogenic acid, one of the most powerful free radical scavengers found in plants. Chlorogenic acid has been shown to decrease low-density lipid (LDL) levels, and also serves as an antimicrobial, antiviral, and anticarcinogenic agent. Polyphenols in eggplant have been shown to exhibit anti-cancer effects. They protect cells from damage caused by free radicals and, in turn, prevent tumor growth and the invasion and spread of cancer cells. Wild species of any crops are considered as a source of a resistant gene to certain pest and diseases, so as found in eggplant. Therefore, it would be the reliable source for resistant gene and desirable characters for future crop improvement.

Despite eggplant's economic importance (FAO ranks it 25th in top commodities), development of molecular genetic resources specific to the species has been limited as compared to other solanaceous crops. So the development of new eggplant varieties addressing old and new breeding objectives (Barchi *et al*., 2012; Lebeau *et al*., 2013; Sunseri *et al*., 2003) requires of genetic diversity. Therefore, collection and characterization of genetic resources are required for the improvement of new varieties. In this respect, molecular characterization is more reliable than morphological characterization (Li *et al*., 2010). Cytoplasmic DNA analysis (Sakata *et al*., 1991; Sakata and Lester, 1997) was the first step of research work corresponding to eggplant but, later experiment replaced with more abundant and polymorphic markers like as Restriction Fragment Length Polymorphism (RFLP) markers (Isshiki *et al*., 1998; Isshiki *et al*., 2003; Doganlar *et al*., 2002a) and random amplified polymorphic DNA (RAPD) markers (Karihaloo *et al*., 1995; Nunome *et al*., 2001; Kashyap *et al*., 2003). SSR markers for eggplant have been developed in the recent years and are being mainly used for assessing the genetic diversity and genome similarity in the related species (Nunome *et al*., 2003; Stàge *et al*., 2008; Nunome *et a*l., 2009; Fukuoka *et al*., 2012). Dominant markers such as RAPDs and AFLP have

been applied to *Solanum melongena* and were proven to be a suitable tool for assessing genetic diversity (Mace *et al.*, 1999b). However, co-dominant markers such as simple sequence repeat (SSRs) could generate more information and has high repeatability than dominant markers. SSRs have proved as a more powerful marker than AFLPs to study the relationships amongst closely related eggplant materials (Munoz-Falcon *et al.*, 2009b).Co-dominant markers are multi-allelic, highly abundant, are well distributed in the genome, and are suitable for high throughput PCR which makes them ideal for diversity studies (Powell, 1996).

Genetic diversity assessment is very important to identify groups with similar genotypes and to conserve, evaluate and utilize the genetic resources. The diversity of the germplasm can be used as a potential basis of genes that lead to improved performance of the superior cultivars and can also be used to determine distinctness and uniqueness of the phenotypes and the genotypes with the objective of protecting the intellectual property rights of the breeder. Wild species remain largely unexploited for eggplant breeding. Detailed phenotypic characterization of wild species and their hybrids with eggplant may allow identifying promising wild species and information on the genetic control and heterosis of relevant traits.

So, the study is focused on both the phenotypic analysis and genetic diversity of eggplant germplasm through co-dominant SSR marker to generate more information and to assess relatedness among local landraces and also with their wild relatives.

With some consideration, the main objectives of this studies were:

1. To investigate phenotypic variation among different local eggplant germplasm.
2. To assess genetic diversity and relatedness among local eggplant germplasm and wild relatives using SSR markers.
3. To identify the genetic distance among the genotypes.

Chapter Two

REVIEW OF LITERATURE

Genetic diversity assessment is an integral part of selecting a highly productive species. It is an essential component in germplasm characterization and conservation. Eggplant surely has a vast genetic diversity and to investigate that molecular markers have been generally superior to morphological, pedigree, heterosis and biochemical data (Melchinger *et al*., 1991). Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level (Weir, 1990). Molecular Marker-based Genetic Diversity Analysis (MMGDA) also has a potential for assessing changes in genetic diversity over time and space (Duwick, 1984).

Kaushik *et al*. (2016) performed an experiment with 6 cultivated accessions, 21 accessions of 12 wild species and 45 interspecific hybrids of eggplant. Significant differences were observed among cultivated, wild and interspecific hybrid groups. Wild species were generally more variable than cultivated accessions and interspecific hybrids. Displayed intermediate ranges of variation and coefficient of variation (CV) values, except for fruit shape traits in which the latter were the most variable. The multivariate principal components analysis (PCA) reveals a clear separation of wild species and cultivated accessions.

Frary *et al*. (2003) studied 58 plants for 207 restriction fragment length polymorphism (RFLP) markers and phenotyped for 18 characters. One to eight loci were detected for each trait. Wild alleles that were agronomically superior to the cultivated alleles were identified for flowering time, flower and fruit number, fruit set, calyx size and fruit glossiness. These results support the mounting evidence of conservation of gene function during the evolution of eggplant and its relatives from their last common ancestor and indicate that this conservation was not limited to domestication traits.

The characterization of wild species and interspecific hybrids for traits of interest for breeders is a fundamental step for the efficient utilization of crop wild relatives in breeding. Combined data on the cultivated and wild species and their interspecific hybrids, not only allows identifying sources of variation and materials of potential

interest, but also provides information on the inheritance of some traits present in the wild species, as has been demonstrated in crosses between *S. incanum* and eggplant (Prohens *et al*., 2013).

2.1 Morphological markers

The knowledge of genetic diversity and its relatedness in the germplasm is a prerequisite for crop improvement programs. Traditionally, characterization of germplasm collections was based primarily on the morphological descriptors which include phenotypic characteristics like flower color, leaf area, leaf length, growth habit etc.

Morphological analysis is the easiest and least complex of the plant identification and characterization techniques. The technique involves description and monitoring of easily detectable parts like form and structure. Several studies have assessed the genetic diversity on the basis of dissimilarity in morphological and agronomic characteristics or on ancestry information for different crops (Liu *et al.,* 2004).

Even though genetic diversity assessment based on morphological characters alone may not be an effective method. The morphological parameters may be used in conjunction with other methods. The diversity based on phenological and morphological characters typically varies with environment. Studies performed on characteristics like leaf blade length, width and shape of leaf blade, thickness and distribution of the lateral and middle shoots and size and shape of flower clusters to distinguish cultivars (Badenes *et al*., 2000; Vinayak *et al*., 2009). Evidence showed that even in modern times the cultivars are illustrated through traits like leaf blade length, leaf blade width, seed weight, seed number, flesh/seed ratio, fruit weight, size, shape and colour (Ntundu *et al*., 2006; Kumar *et al.,* 2007; Antonius *et al.,* 2011; Santos *et al.,* 2011; Blanckaert *et al.,* 2012).

Halsted *et al.* (1918) compared the inheritance of the fruit colors of tomato, eggplant and pepper and noticed genetic similarities between them, which might be considered as the first observation of synteny. So, an accurate identification becomes difficult in the process, lowering the reliability of morphological markers for germplasm characterization.

But studies also indicated that conventional methods for characterization and evaluation of genetic variability, based on morphological and physiological studies, are time consuming and influenced by the environment (Nicese *et al*., 1998 and Mondini *et al.*, 2009).

2.2 Molecular markers

Molecular markers have been found to be more dependable than the phenotypic observations for evaluating the variations and in the assessment of the genetic stability (Leroy *et al.*, 2000) and provide an efficient means to link phenotypic and genotypic variation (Varshney *et al*., 2005).

These methods are being very rapidly adopted by the researchers all over the world for the crop improvement. The molecular marker techniques are diverse and vary in principle, application and amount of polymorphism observed and in time requirements (Vilanova *et al.*, 2012). Molecular markers present an efficient tool for fingerprinting of cultivars, and assessment of genetic resemblance and relationships (Vilanova *et al.*, 2012).

A number of molecular markers have been developed and employed in the analyses of genetic diversity and relatednes. They are classified as: (i) hybridization based markers i.e. restriction fragment length polymorphisms (RFLPs), (ii) PCR-based markers i.e. random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), inter simple sequence repeats (ISSRs) and microsatellites or simple sequence repeats (SSRs), and (iii) sequence based markers i.e. single nucleotide polymorphisms (SNPs) (Varshney *et al*., 2007).

Li *et al.* (2010) stated that molecular characterization is more reliable than morphological characterization. They 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 means based on similarity matrices formed three different clusters. 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.

Among the available molecular markers, microsatellites or simple sequence repeats (SSRs) which are tandem repeats of one to six nucleotide long DNA motifs, have gained considerable importance in plant genetics and breeding owing to many desirable genetic attributes including hypervariability, multiallelic nature, codominant inheritance, reproducibility, relative abundance, extensive genome coverage including organellar genomes, chromosome specific location and amenability to automation and high throughput genotyping (Kalia *et al*., 2011).

2.3 Microsatellite or Simple Sequence Repeats (SSRs) marker

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

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

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

SSR markers are non-isotype based precise PCR technology. Stretches of DNA, consisting of randomly repeating small nucleotide units and conserved regions flanking the repeats are suitable for designing PCR primer pairs. Used for amplifying

the intervening repeat loci were first referred to as Microsatellites by Litt and Luty (1989) and later as Simple Sequence Repeats (SSRs) by Jacob *et al*. (1991).

Cericola *et al*. (2013) phenotyped 238 eggplant breeding lines, heritage varieties and selections within local landraces from Asia and the Mediterranean Basin with respect to key plant and fruit traits, and genotyped using 24 microsatellite loci distributed uniformly throughout the genome.

Bryan *et al*. (1999) developed some PCR-based markers from mononucleotide simple-sequence repeats in the chloroplast genome of *Nicotiana tabacum* and applied to the analysis of genetic diversity and were found to detect high levels of polymorphism at three taxonomic levels in Solanaceous plants.

Smith *et al.* (1997) made a comparison of SSR with data from RFLP and pedigree in maize. They stated that SSR revealed co-dominantly inherited multi-allelic product of loci that can be readily mapped. SSR profiles can be interpreted genetically without the need to repeatedly map amplified bands to marker loci in the different populations. They anticipated that SSR profiling will replace RFLP and PCR based arbitrary primer methods. Munoz-Falcon *et al*. (2011) have shown that genomic SSR markers are more polymorphic than EST-SSRs in eggplant.

On the other hand, SSRs provide a powerful means to link the genetic maps of related species, and since many of them are located within genes of known, any allelic variation present can be exploited to generate perfect markers (Andersen et al., 2003). So transfer of SSR markers is a very efficient approach for DNA marker development.

2.4 SSR markers in molecular diversity analysis in Eggplant

Though eggplant is the most desirable vegetable in the world regarding its taste and availability, experiment concerning improvement and resistance was not performed as expected comparing with other Solanaceous species. Very few experiments of eggplant using molecular markers have been found.

Demir *et al.* (2010) performed an experiment on eggplant genotypes collected from different geographical regions of Turkey using SSR and RAPD markers. A total of 24 alleles were amplified for each of 5 microsatellite locus with a ranged from two to ten. The highest number of alleles was recorded 10 at the emf21H22 locus was followed by emh11O01 and emf21C11 as 5 and 4s alleles, respectively with an average of 4.8. 100 bands among which 29 were polymorphic were amplified for eleven RAPD markers. The number of bands per primer ranged from 7 to 14. Primer OPB07 was the most polymorphic, generating 64% polymorphic bands; the rest of the primers gave less than 50% polymorphism. UPGMA dendrogram were used to examine the genetic relatedness of the genotypes.

Muñoz-Falcón *et al.* (2011) studied 42 eggplant accessions, which included 25 *Striped* accessions, of which 19 were of the *Listada* type (renowned spanish eggplant) and 6 of the *Other Non-Spanish Striped* group and 17 *Non-Striped* accessions were characterized with 17 genomic SSRs and 32 EST-SSRs. Genomic SSRs had a greater polymorphism and polymorphic information content (PIC) than EST-SSRs. *Listada de Gandía* proved to be genetically diverse, specific and universal alleles for two SSR markers were found for this landrace. All the *Listada* accessions cluster together in the multivariate PCoA and UPGMA phenograms performed, and are separated from the *Other Non-Spanish Striped* and *Non-Striped* accessions. SSR markers revealed of great utility to obtain a specific fingerprint for the *Listada de Gandía* eggplant as well as to establish the uniqueness and distinctness of this landrace.

Ge *et al*. (2011) observed that a random set of 100 EST-SSR primers were amplified in 12 eggplant accessions and 88 successfully amplified expect PCR products. 32 markers revealed 83 polymorphic alleles among the 42 cultivated accessions and the number of allelles per locus varied between 2 and 6 (mean 2.6). Polymorphism information content (PIC) values among the 42 cultivated types were calculated and varied from 0.045 to 0.701 (mean 0.289). The markers showed low frequency transferability in Solanaceae. The 32 SSRs were used to evaluate genetic diversity. These SSRs will be valuable markers for future genetic study, such as genetic diversity estimation, linkage mapping, association mapping and molecular breeding.

Tümbilen *et al.* (2011) identified genic microsatellite (SSR) markers from an expressed sequence tag library of *S. melongena* and used for analysis of 47 accessions of eggplant and closely related species. The markers had very good polymorphism in the 18 species tested including 8 *S. melongena* accessions. Moreover, genetic analysis performed with these markers showed concordance with previous research and knowledge of eggplant domestication. These markers are expected to be a valuable resource for studies of genetic relationships, fingerprinting, and gene mapping in eggplant.

Ali *et al.* (2011) analyzed the 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. Their ISSR/RAPD data provide molecular evidence that coincides with morphological-based classification into three varieties and further subdivision into eight groups, except for two groups. 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.

Caguiat *et al.* (2012) showed the first report on genetic diversity assessment of Philippine eggplant accessions and landraces, six wild species and six cultivated variaties among the traditional varieties (improved cultivars and hybrids) were characterized using 17 simple sequence repeat (SSR) markers. The results showed high genetic variation among the traditional varieties and landraces of *S. melongena*. In contrast, low genetic variation was observed among the cultivated varieties and hyrids. Low genetic variation was also observed among the wild species analyzed using SSRs markers. This result is in contrast to the high genetic variation observed among the wild species used using morphological traits. Among the three groups of eggplant genetic resources analyzed, the traditonal eggplant and landraces were the most diverse. Implications of these findings for eggplant breeding programs and germplasm management were discussed.

Hurtado *et al.* (2012) have assessed the diversity and relationships of 52 accessions of eggplant from three geographically distant secondary centers of diversity (China,

Spain, and Sri Lanka) using 28 morphological descriptors and 12 highly polymorphic genomic SSRs. A wide variation was found for most morphological traits, and significant differences among the three centers of diversity were detected for 22 of these traits. The PCA analysis showed that eggplants from the three origins were morphologically differentiated and surely revealed diversity. The genetic diversity (H_T) within each origin was high, ranging between H_T=0.5400 (Sri Lanka) and H_T=0.4943 (China), while the standardized genetic differentiation (G'_{ST}) among origins was moderate (G'_{ST}=0.2657).

Adeniji *et. al.* (2012) conducted a research on 7 *Solanum* species (eggplants) to investigate genetic diversity. Simple sequence repeat (SSR) markers were used on 39 *Solanum* genotypes, a landrace and tomato variety (LBR 48) to observe their performance based on the Jaccard's coefficient of similarity and UPGMA clustering. 417 alleles were amplified with the quantity of alleles running from 5 (EM 141) to 38 (EM 120 b). Polymorphism was genuinely high (0.05 to 0.92) among SSR markers with high number of repeats.

Chinnappareddy *et al*. (2012) conducted an experiment at Indian Institute of Horticulture Research (IIHR) on eggplant breeding program produced five genotypes with increased yield and excellent producer acceptance. Molecular profiles of these genotypes were developed using 39 EST-SSR primers for maximum discrimination and repeatability at 35 loci. Polymorphic information content of the markers ranged from 0.343 to 0.794. Expected heterozygosity ranged from 0.560 to 0.880 and the probability of identity ranged from 0.010 to 0.376. Pair-wise comparison of microsatellite data led to development of an unweighted pair group method with arithmetic mean dendrogram and DNA barcodes for easy and accurate identification of eggplant genotypes with combinations of their morphological traits. The results indicate that 'IIHR-3' and 'IIHR-7' originated from a single source and 'Arka Anand' is a cross between 'IIHR-3' and 'SM-6-6', indicating a good fit with genetic similarity values.

Ge *et al.* (2013) conducted an experiment in where 100 simple sequence repeat (SSR) markers were used to examine the genetic diversity and relationships among 92 eggplant accessions collected from seven areas in China. These analysis 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 results will be useful for eggplant germplasm management and will lead to more efficient use of germplasm in eggplant breeding.

Vilanova *et al.* (2014) used 19 genomic SSRs for the molecular characterization of 30 eggplant accessions corresponding to the four cultivar groups. The polymorphism information content (PIC) of SSR markers ranged from 0.07 to 0.77, with an average value of PIC=0.50. The mean observed heterozygosity (Ho) presented a very low value Ho=0.01, while the mean expected heterozygosity (He) had a value of He=0.57.

Kumar *et al.* (2014) performed a study to identify the SSR markers that could be used to test the genetic purity of three popular eggplant hybrids (*viz.*, PH-5, PH-9 and Kashi Komal). Among 30 SSR markers 6 were found to be suitable for testing the purity of these hybrids. The analysis of plant-to-plant variation within the parental lines of all the hybrids, using the identified hybrid specific markers, showed highly homogenous SSR profile, which further indicated the scope of application of these markers in maintenance and purity testing of hybrids and parental lines.

Caguiat *et al*. (2014) showed that 33 out of the 41 SSR primer pairs (80.48%) detected variation among the accessions and the number of alleles ranged from 2 to 8 with a mean of 4.3 alleles per marker. The morphological trait and SSR data were analyzed as separate and combined data sets using principal component analysis (PCA) and unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis. Similar results were obtained from the 3 data sets. Landraces, cultivars and crop wild relatives (CWR) were clearly differentiated. CWRs were the most diverse group, followed by the landraces, while the improved cultivars were the least diverse. This study provided significant information for the need to increase the present

eggplant collection and to widen the genetic diversity of currently cultivated eggplant varieties in the Philippines.

Boyaci *et al.* (2015) studied over 38 eggplant genotypes, of which 32 were heirloom accessions collected from different regions of Burdur province five were different local genotypes from other provinces, and one was a cultivar, were used as reference in this study. The phylogenetic relationships among these heirlooms were evaluated using 40 morphologic descriptors and five randomly amplified polymorphic RAPD markers. The horizontal dendrograms were created by using UPGMA with both morphologic and molecular data. Burdur heirloom accessions showed high genetic diversity based on morphological and molecular data. The genetic similarity rates ranged from 0.29 to 0.91 according to the morphological data, and ranged from 0.84 to 0.98 according to the molecular data. Molecular data generated by RAPD method, compared to morphological data, were insufficient to reveal genetic diversity.

Therefore, in order to confirm genetic variations, studies based on other molecular methods are necessary. The regional genetic populations include a wide eggplant genetic diversity which can be good source for the breeding studies performed in the future.

From the previously mentioned literature, it is undoubtedly found that SSR examination is exceptionally proficient for the genetic diversity analysis for local and wild species of eggplant. The present review is intend to decide genetic distance, similarities, resistances and relatedness within 25 local eggplant germplasm and 2 wild species of Bangladesh using microsatellites or SSR markers.

Chapter Three

MATERIALS AND METHOD

The chapter constitutes the materials and methods used in the experiment. The details of the methodology have been followed is described below:

3.1 Experimental site and time Duration:

The experiment was carried out in two different aspects. One was morphological analysis and another was molecular-based analysis. To achieve the objectives experiment was conducted on the field and biotechnology lab of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207. The experiment was carried out during the period of October 2015 to July 2016. Two experiment was conducted to fulfill the mentioned objectives.

Sub-experiment 1. Morphological characterization of different local eggplant genotypes and wild relatives

Sub-experiment 2. Molecular diversity analysis of local eggplant germplasm and wild relatives

3.2 Materials and methods for sub-experiment 1

3.2.1 Collection of material

A total of 27 eggplant germplasm accessions were used in this experiment. Among those 25 were local landraces and 2 were wild relatives. Germplasms were collected from different districts of Bangladesh. A list of germplasm collected area was given in Table 1. Wild relatives were collected from the Gene Bank of Bangladesh Agricultural Research Institute (BARI), Gazipur.

Table 1. Name of the germplasm and collected area

SL. No.	Entry Name	Collected Area
1	Salta Begun	Lalmonirhat District
2	Ashary	Lalmonirhat District
3	Lalmoni Local-1	Lalmonirhat District
4	Lalmoni Local-2	Lalmonirhat District
5	Kurigram Local	Kurigram District
6	Khotkhotia	Rangpur District
7	Cricket	Rangpur District
8	Rangpur Local-1	Rangpur District
9	Rangpur Local-2	Rangpur District
10	Rangpur Local-3	Rangpur District
11	Nilphamari Local	Nilphamari District
12	Dinajpur Local	Dinajpur District
13	Thakurgaon local	Thakurgaon District
14	Bogra Local	Bogra District
15	Iswardi Local	Pabna District
16	Jessore Local-1	Jessore District
17	Jessore Local-2	Jessore District
18	Jessore Local-3	Jessore District
19	Jessore Local-4	Jessore District
20	Sada Khulna	Khulna District
21	Khulna Local-1	Khulna District
22	Jamalpur Local	Jamalpur District
23	Narsingdi Local	Narsingdi District
24	Comilla Local	Comilla District
25	Dohazari	Comilla District
26	Wild species (*Solanum sisymbriifolium*)	BARI, Gazipur
27	Wild species (*Solanum torvum*)	BARI, Gazipur

3.2.2 Germination test

Seed viability was tested in Petri dish. It was observed that the highest (95%) and the lowest (30%) seed germination was recorded. Seeds were shown in the middle of October 2015. Seedlings were germinated within a week. After few days seedlings were transplanted to the main field and the experiment was designed in RCBD with 2 replications.

3.2.3 Morphological character assessment

Observations were made on morphological characters in different growth and development stages. The experiment was conducted in a Randomized Complete Block Design with two replications. Plant to plant distance kept 50 cm × 50 cm and plot to plot distance maintained 100 cm × 100 cm. The seeds were sown in a tray in October 2015. 20 seeds were sown in each tray with a mixture of compost and soil. The plants were transferred to experimental plots after three weeks of sawing, and the required irrigation and fertilizer were applied. Each plot contained different types of genotypes. The fruits were harvested at maturity. The morphological characterization data of 25 local eggplant germplasm and 2 wild races were obtained through regular observation. Morphological data was recorded on the basis of both qualitative and quantitative traits. The morphological traits included stem color, no. of branch per plant, days to 50% flowering, peel color of fruit, fruit length (cm), fruit diameter (cm), calyx color, single fruit weight(g), general impression (scale 1-5), leaf vein color, fruit shape, presence of spine, yield per plant (kg), yield per plot(kg). The quantitative and qualitative data were measured in 10 samples of plant and fruits of per accession.

3.2.4 Morphological data analysis

Morphological data was analyzed through using Statistical Tool for Agricultural Research (STAR) compatible version 2.0.1 and Plant Breeding Tools (PBTools), Version: 1.3. Pairwise mean differences were tested according to Tukey's Honest Significant Difference (HSD) Test. The correlation was estimated following Pearson’s product moment correlation analysis.

3.3 Materials and methods for sub-experiment 2

3.3.1 Collection of leaf sample

In order to carry out SSR marker analysis, young, fresh and tender leaves from the seedlings of about one-month-old were collected from each genotype and used as the source of genomic DNA extraction.

3.3.2 Genomic DNA extraction

DNA extractions from young leaves were performed according to a modified Doyle and Doyle (1990) method by using CTAB protocol. The following reagents and methods were used for the isolation of total genomic DNA.

3.3.3 Reagents required

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
- 0.5 M EDTA
- D. H_2O

4. Isopropanol

5. 0.3 M Sodium Acetate

6. Absolute (100%) ethanol

7. Ethanol (70%)

8. RNAse

9. Ethidium Bromide Solution

3.3.4 Reagent preparation for DNA extraction

Extraction buffer (1000ml)

For the preparation of 1000ml DNA extraction buffer, 100ml 1M Tris HCL (pH =8.0) was mixed with 40ml of 0.5M EDTA and added to 100ml 5M NaCl in a 1000ml measuring cylinder. Finally, sterilized dH_2O was added to make the volume up to 1000 ml, then mixed well and autoclaved.

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

Initially, 30.28 g Tris was taken in a volumetric flask (500 ml). 100ml dd H_2O was added and pH was adjusted to 8.0 by adding HCl. Then sterilized dH_2O was added to make the volume up to 250 ml. The solution was autoclaved.

0.5M EDTA (pH 8.0) (250ml)

46.53 g EDTA.$2H_2O$ was added in a volumetric flask (500 ml) and 100ml dd. H_2O was added. Then 4 g NaOH was added and pH was adjusted to 8.0 with NaOH. Sterilized dH_2O was added to make its volume up to 250ml. Finally the solution was autoclaved.

5M NaCl (250ml)

For the preparation of 5M NaCl, 73.05 g of NaCl was added in 250 ml dd. H_2O in a 500 ml volumetric flask, mixed well and autoclaved.

1% SDS (Sodium Dodecyl Sulphate) (100 ml)

1g of SDS was added in 100ml dH_2O in a 250 ml beaker.b As SDS is hazardous, so the proper mixing was done by a hot top magnetic stirrer and was not autoclaved.

2 X CTAB (for plant DNA extraction)

To make 2% CTAB solution, 2g of CTAB was added in 100ml 0.5 M NaCl. Finally the solution was autoclaved.

1X TE buffer (100ml)

1ml Tris (pH 8.0) was taken in a volumetric flask (250ml). Then 0.2ml EDTA (pH 8.0) was added. Sterilized dH_2O was added to make the volume upto 100ml.

Composition of 5X TBE buffer (1 litre)

- 54 g Tris HCl
- 27.5 g of Boric Acid
- 4.65 g of EDtA
- pH=8.0

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

At first 50ml phenol was taken in a volumetric flask (250ml). Then 48ml Chloroform was added. 2ml Isoamyl Alcohol was also added and mixed well. The solution was stored at 4^oC.

RNase

10mg of RNase was added to 1 ml of d. H_2O. Then it was dissolved completely with the help of necessary heat (at 50^oC in water bath for 30 minutes).

70% Ethanol (1000 ml)

700 ml absolute ethanol was mixed with 300 ml dH_2O**.**

3.3.5 Sequential steps for DNA extraction

1. For Isolation of genomic DNA, vigorous, young, actively growing fresh leaf tissues were collected from 27 different eggplant germplasm. Total DNA was isolated by using Phenol: Isoamyl Alcohol and ethanol precipitation method.

2. Initially, healthy youngest leaves were washed thoroughly by tap water followed by deionized water. Then sterilized by ethanol to remove wastes and any source of foreign DNA and leaves are then dried on tissue paper.

3. Approximately 200mg of young leaves were cut into small pieces and then taken in morter. 600µl of extraction buffer was added to it. The ground samples were taken into the 1.5ml eppendorf tube and then it was vortexed for 20 seconds in a vortex mixture and then incubated at 65^0C for 20 minutes in hot water bath.

4. Equal volume (600 µl) of Phenol: Chloroform : Isoamyl Alcohol (25: 24 : 1) was added to the tube. Then it was vortexed for 20 seconds.

5. The solution was then centrifuged for 10 minutes at 13000 rpm. The supernatant was recovered using a pipette tip without disturbing the lower portion and transferred into a new eppendorf tube. Approximately 400-450 µl was taken and then equal volume of Chloroform : Isoamyl Alcohol (24: 1) was added to it. The solution was vortexed for 10 seconds.

6. Again the solution was centrifuged at 13000 rpm for 10 minutes.

7. The supernatant was taken in a separate eppendorf tube and the lower layer was discarded.

8. The amount of the solution was multiplied with 0.6 and then same volume of Isopropanol (0.6 volume of the liquid) was added.

9. It was then tapped by finger for 20-30 seconds (The genomic DNA was visible as cotton like structure).

10. 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 20 µl 0.3 M Sodium acetate.

11. 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.

12. The DNA pellet was then air dried for 2-3 hours. It was then dissolved in an appropriate volume (30 to 40 µl) of TE buffer and treated with 3 µl of RNAse for removing RNA. Then it was spinned for 4-5 seconds.

13. Finally, the DNA samples were stored in freezer at -20^{o}C.

3.3.6 Confirmation of extracted DNA

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

Table 2. DNA confirmation reagents with amount

Components	Amount (µl)
Working DNA sample	2.0
D. H_2O	3.0
2X loading dye	3.0
Total	8.0

3.3.7 Working solution of DNA samples preparation

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

Where,

V_1 = Initial volume of DNA solution (µl)

S_1 = Initial DNA concentration (ng/µl)

V_2 = Final volume of DNA solution (µl)

S_2 = Final DNA concentration (ng/µl)

3.3.8 1% Agarose gel preparation

Reagents

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

Procedure

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

solution was cooled to about 45-50^{0}C (flask was cool enough to hold comfortably with bare hand) and 3µl 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 (15×15×2 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 25 minutes, gel was completely cooled at room temperature and solidified and the comb was removed gently. The gel was then ready for loading the DNA samples.

3.3.9 Preparation of DNA samples for electrophoresis

The samples were all in the same concentration in buffer. For each sample, 3 µl dH_2O and 3µl 2X loading dye (0.255 xylene ethanol, 0.255 bromophenol blue, 30% glycerol and 1mM EDTA) was taken in an eppendorf tube using 0.5-10µl adjustable micropipette. Loading dye was used for monitoring loading and the progress of the electrophoresis and to increase the density of the sample so that it stayed in the well. Finally, 2µl expected 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 (runnig buffer). The gel tank was covered and the electrophoresis power supply was connected and turned on to move DNA from negative to positive (black to red) through the gel. Electrophoresis was carried out at 80V for about 50 minutes.

3.3.10 Documentation of the DNA samples

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

3.4 Amplification of SSR markers by PCR

3.4.1 Principle of the amplification of SSR marker

Microsatellites or SSR are tandem repeats of 1 – 6 nucleotides. For example, $(A)_n$, $(AT)_n$, $(ATG)_n$, $(GATT)_n$, $(CTACG)_n$, $(TACGAC)_n$, and so on. They are abundant in genomes of all organisms. The sequence of unique flanking regions of SSR can be used to design primers and carry out PCR to amplify SSR containing sequences. The polymorphism can be detected by agarose gel electrophoresis if differences are large enough (agarose gels can detect differences greater than 10 base pair). SSRs were first used as markers for use in genetic mapping in humans (Litt and Luty, 1989).

3.4.2 List of SSR primers

Five well known SSR primers *viz.* EM114, EM120, EPSSR82, SMSSR01, EPSSR04 were selected for PCR reaction on 25 local and 2 wild relatives of eggplant germplasm for their ability to produce polymorphic band.

Table 3. The list of SSR primers and their sequences and GC content

Name of SSR primer	Sequences of the primer (5'-3')	% of (G+C) content	Expected band sizes (bp)	Annealing tempetarure (0^oC)
EM114	For. AGCCTAAACTTGGTTGGTTTTTGC Rev.GAAGCTTTAAGAGCCTTCTATGCAG	43	234- 246	65
EM120	For. GGATCAACTGAAGAGCTGGTGGTT Rev.CAGAGCTTCAATGTTCCATTTCACA	44	160-164	65
EPSSR82	For. ACATGCCACTCATGTTGGTG Rev. CTTCAGCCATGGACCACATT	50	140-250	64
smSSR01	For. GTGACTACGGTTTCACTGGT Rev. GATGACGACGACGATAATAGA	46	180-270	65
smSSR04	For. AATGAGTCAGAAACCACGCC Rev. CGTTTAACCTTTGGCTCGGAA	49	155-250	63

3.4.3 PCR amplification and reactions

PCR reactions were performed on each DNA sample. 2X Taq ready Master Mix was used. DNA amplification was per formed in oil-free thermal cycler (Esco Technologies swift^{TM} Mini Thermal Cyclers). To prepare a 10 µl reaction mixture containing ready mix Taq DNA polymerase and other compositions were given in Table 4.

Table 4. Reaction mixture composition for PCR for each eggplant genotype

Reagents	**Amount(µl)**
2X Taq Master Mix	5.00
SSR Forward primer	0.75
SSR Reverse primer	0.75
De-ionized water	2.50
Sample DNA	1.00
Total Reaction volume	10.00

From frozen stocks of the PCR reagents i.e., 2X Taq Master Mix, primer and DNA working samples were melt, mixed by vortexing and kept on ice for maintaing good quality. DNA samples 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 mixture, DNA sample and de-ionized water. Then the mixture was mixed up well and aliquoted into the tubes containing primer. The tubes were then sealed and placed in a thermo cycler and the cycling was started immediately.

3.4.4 PCR amplification thermal profile

DNA amplification was performed in a thermal cycler (Esco Technologies Swift^{TM} Mini Thermal cyclers). Polymerase chain reaction (PCR) technique is used to selectively amplify a specific segment of the total genomic DNA based on the selected SSR primers. SSRs were amplified under the following PCR reaction conditions: Pre-denaturation with 95◦C for 4 min; denaturation with 95◦C for 40 sec, annealing at 61◦C for 33 sec, extension at 72^{o}C for 40 sec ,final extension at 72^{o}C for 5min continuing with 31 cycles and finally stored at 4^{o}C.

3.4.5 Electrophoretic separation of the amplified products

PCR products for each sample were confirmed by running it in 2% agarose gel containing 1µl ethidium bromide in 1X TBE buffer at 90 V for 1 hour. 5 µl Loading dye was added to the PCR product and spinned them well. Then loaded them in the wells and 100 bp DNA ladder (Promega) was also placed in both left and right side of the gel. Under ultra-violet light on a trans-illuminator SSR bands were observed.

3.4.6 Documentation of PCR amplified DNA products:

The gel was taken out carefully from the gel chamber and was placed on high performance ultra-violet light box (UV trans-illuminator) of gel documentation for checking the DNA band and photographed by a Gel Cam Polaroid camera.

3.4.7 SSR data analysis:

The summary statistics including the number of alleles per locus, major allele frequency, gene diversity and Polymorphism Information Content (PIC) values were determined using POWERMARKER version 3.25 (Liu K. & Muse SV. 2005), a genetic marker data analysis software. Molecular weight for each microsatellite products, in basepairs were estimated with AlphaEaseFC (Alpha Innotech Corporation) version 4.0 software. The individual fragments were assigned as alleles

of the appropriate microsatellite loci. The allele frequency data from POWERMARKER was used to export the data in binary format (presence of allele as '1' and absence of allele as '0') for analysis with NTSYS-PC (Numerical Taxonomy and Multiware Analysis System) version 2.2 software (Rohlf 2006) . Unweighted Pair Group Method of Arithmetic Means (UPGMA) dendrogram was construcred using a computer programme, POPGENE (Version 1.31) based on Nei's (1972) genetic distance.

3.5 Precautions

- To maintain a strategic distance from all types of contaminations and keep DNA pure, all dishes, micropipette tips, eppendorf tubes, glass pipettes, de-ionized water and buffer solutions were legitimately autoclaved. Metal supplies i.e., scissors, forceps were cleaned with absolute ethanol.
- Since Ethidium Bromide (Et-Br) is an intense mutagen and carcinogenic in nature, hand gloves were utilized when taking care of anything that has been presented to Et-Br.
- Always power pack was kept turn off and the leads were unplugged before opening the lectrophoresis unit to avoid electrical hazard.
- Eye protector was used while working with trans-illuminator as it produces UV radiation of 254 nm range which can cause eye damage.
- The common safety measures were kept up when performing PCR responses. All the 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 amid treatment of PCR segments. Contamination of PCR segments was maintained a strategic distance from.

Chapter Four

RESULTS AND DISCUSSION

Eggplant (*Solanam melongena*) is an old world species complex that includes weedy and wild relatives as well as primitive cultivars and landraces. The molecular genetic maps developed in eggplant have been used both for the tagging of simply inherited traits and the localization of the loci underlying complex morphological characters. The assessment of genetic diversity or relatedness is not only important for eggplant improvement but also for the conservation and maintenance of germplasm. The experiment was carried out to study the molecular diversity of eggplant local genotypes and its two wild relatives. The key finding was given below:

4.1 Sub-experiment 1: Morphological characterization of different local eggplant genotypes and wild relatives

Twenty five local eggplant germplasm and two wild species were characterized as per the description of phenotypic characterization. The following important morphological traits were studied.

4.1.1 Color of leaf vein

Color of leaf vein among different genotypes of eggplant displayed distinct variation (Table 5). The genotypes Rangpur local-1, Nilphamari local, Jessore local-1, Sada Khulna, Khulna local-1 and two wild speces *Solanum sisymbriifolium* and *Solanum torvum* exhibited green color. The materials Jessore local-3 and Jessore local-4 showed pink color and rest of the genotypes exhibited purple color.

4.1.2 Stem color

Stem morphology of all experimental materials showed purple and green color. Among them eighteen genotypes were purple in color and rest were green (Table 5).

4.1.3 Fruit color

A diversified color was found on fruits appearance in various genotypes. Among them purple, deep purple, light purple, green, light green were more common. Two wild

species *Solanum sisymbriifolium* and *Solanum torvum*, Narsingdi local, Rangpur local, Jessore local-1, Sada Khulna, Khulna local-1 and Dohazari exhibited green color, and rest of all showed deep purple to light purple in color (Table 5).

4.1.4 Fruit Shape

The twenty five eggplant genotypes showed tremendous variation in fruit shape like long, round, oval and oblong. But, two wild species *Solanum sisymbriifolium* and *Solanum torvum* produced very small sized fruit (Table 5).

Table 5. Phenotypic characters of 25 local and two wild relatives of eggplant

SL. No.	Genotypes	Color of Leaf vein	Stem color	Fruit color	Fruit shape
1	Salta Begun	Purple	Purple	Deep purple	Long
2	Ashary	Purple	Purple	Purple	Long
3	Lalmoni Local-1	Purple	Purple	Purple	Long
4	Lalmoni Local-2	Purple	Purple	Deep Purple	Long
5	Kurigram Local	Purple	Purple	Purple	Long
6	Khotkhotia	Purple	Purple	Purple	Oblong
7	Cricket	Purple	Purple	Purple	Oval
8	Rangpur Local-1	Green	Green	Green	Long
9	Rangpur Local-2	Purple	Purple	Purple	Oblong
10	Rangpur Local-3	Purple	Purple	Purple	Long
11	Nilphamari local	Green	Green	Light Purple	Long
12	Dinajpur Local	Purple	Purple	Deep Purple	Long
13	Thakurgaon local	Purple	Purple	Purple	Long
14	Bogra Local	Purple	Purple	Purple	Oblong
15	Iswardi	Purple	Purple	Purple	Round
16	Jessore Local-1	Green	Green	Green	Round
17	Jessore Local-2	Purple	Purple	Purple	Oval
18	Jessore Local-3	Pink	Pink	Purple	Oval
19	Jessore Local-4	Pink	Pink	Purple	Oval
20	Sada Khulna	Green	Green	Green	Oval
21	Khulna Local-1	Green	Green	Green	Oval
22	Jamalpur Local	Purple	Purple	Light green	Oblong
23	Narsingdi local	Purple	Purple	Green	Round
24	Comilla Local	Purple	Purple	Deep purple	Oval
25	Dohazari	Purple	Purple	Green	Round
26	Wild species (*Solanum sisymbriifolium*)	Green	Green	Green	Round
27	Wild species (*Solanum torvum)*	Green	Green	Green	Round

Figure 1. Radomly photographed picture of some fruits from studied eggplant genotypes

4.1.5 Fruit morphology

The fruit morphology of the genotypes was presented in Table 6. Each of the genotypes showed individual fruit length, breadth and weight. Fruit length ranged from 0.8 cm (*Solanum torvum*) to 27 cm (Salta begun) and fruit breadth ranged from 0.75 cm (*Solanum torvum*) to 11 cm (Iswardi). The highest fruit weight was observed in Iswardi (411g) followed by Sada Khulna (347g), Khotkhotia (345g) and the lowest was in wild specis *Solanum sisymbrifolium* (10g).

Table 6. Fruit morphology of 25 local eggplant genotypes and 2 wild relatives

SL. No.	Genotypes	Fruit wt.(g)	Fruit length (cm)	Fruit breadth (cm)
1	Salta Begun	104	27.0	3.0
2	Ashary	107	24.0	3.0
3	Lalmoni Local-1	102	26.0	2.5
4	Lalmoni Local-2	105	25.0	3.0
5	Kurigram Local	118	24.0	3.0
6	Khotkhotia	345	20.5	6.5
7	Cricket	142	8.0	5.5
8	Rangpur Local-1	125	26.0	3.0
9	Rangpur Local-2	157	14.5	7.0
10	Rangpur Local-3	82	25.5	2.5
11	Nilphamari local	150	23.0	4.5
12	Dinajpur Local	85	25.0	2.5
13	Thakurgaon local	134	21.0	4.5
14	Bogra Local	111	13.0	4.5
15	Iswardi	411	10.5	11.0
16	Jessore Local-1	141	10.5	9.0
17	Jessore Local-2	332	11.0	8.5
18	Jessore Local-3	291	13.5	8.0
19	Jessore Local-4	285	13.0	8.0
20	Sada Khulna	347	22.0	7.5
21	Khulna Local-1	342	21.5	7.0
22	Jamalpur Local	104	9.5	5.0
23	Narsingdi local	312	11.0	9.5
24	Comilla Local	294	10.0	9.0
25	Dohazari	305	12.0	9.0
26	Wild species (*Solanum sisymbriifolium*)	10	0.85	0.75
27	Wild species (*Solanum torvum)*	12	0.80	0.75

4.2 Yield contributing traits of eggplant genotypes and wild relatives

4.2.1 Days to 50% flowering

Days to 50% flowering of the 27 genotypes ranged from 40 to 68 days. The genotype Iswardi was the earliest, which flowered within 40 days after transplanting and Bogra

local was the late one and it flowered at 68 days after transplanting. Rest of the genotypes flowered within 60 to 65 days after transplanting (Table 7).

4.2.2 Branch per plant

Branch per plant varied from 3 to 8. The highest number of branches per plant as found in wild species (8) that formed a bushy appearance. However, the average number of branch per plant was estimated 5.0 (Table 7).

4.2.3 Yield per plant

Yield per plant was recorded in kilogram (kg). It was found that Salta begun (9.8 kg) and Lalmoni local-1 (9.5 kg) gave the highest yield. The wild spices *Solanum torvum* and *Solanum sisymbriifolium* produced the lowest yield 0.6 kg individually. As wild species produced small size fruits with minimum weight hence it produced the lowest yield (Table 7).

4.2.4 Yield per plot

The genotypes which gave the highest yield per plant was also exhibited similar result for yield per plot. The highest yield per plot was observed in Salta Begun (58.5 kg) and the lowest was observed in the wild species (3.8 kg) (Table 7).

Analysis of variance for four response variables such as (a) days to 50% flowering; (b) branch per plant; (c) yield per plant and (d) yield per plot of the different eggplant genotypes showed significant variations for each of those traits. The results among all the genotypes showed future prospect of this study, as significant means there would have prossibilities for crop improvement in future. ANOVA for above mentioned four traits are shown in **Appendix no. 1.**

Table 7. Major yield contributing traits of eggplant genotypes and its wild relatives

Genotype	**Days to 50% flowering**	**Branch/Plant**	**Yield/Plant (kg)**	**Yield/Plot (kg)**
Ashary	66	6	8.5	51.0
Bogra Local	67	5	3.3	19.5
Comilla Local	56	5	6.7	39.9
Cricket	43	4	6.3	37.5
Dinajpur Local	64	5	7.8	46.5
Dohazari	66	6	5.0	30.0
Iswardi	41	4	3.5	21.0
Jamalpur Local	59	6	7.0	42.0
Jessore Local-1	51	7	6.3	37.5
Jessore Local-2	64	4	7.8	46.5
Jessore Local-3	65	4	6.8	40.5
Jessore Local-4	65	4	7.5	45.0
Khotkhotia	47	4	7.8	46.5
Khulna Local-1	64	5	6.5	39.0
Kurigram Local	45	6	9.0	54.0
Lalmoni Local-1	61	4	9.5	57.0
Lalmoni Local-2	60	6	8.5	51.0
Nilphamari local	43	6	9.5	57.0
Narsingdi local	67	5	5.3	31.5
Rangpur Local-1	68	4	4.3	25.5
Rangpur Local-2	46	3	6.3	37.5
Rangpur Local-3	50	5	7.0	42.0
Sada Khulna	65	4	6.3	37.5
Salta Begun	68	5	9.8	58.5
Thakurgaon local	56	6	4.8	28.5
Wild species (*Solanum torvum)*	62	8	0.6	3.80
Wild species (*Solanum sisymbriifolium*)	65	8	0.6	3.80
Mean	**58.11**	**4.94**	**6.35**	**38.13**
Heritability (%)	**98.00**	**90.00**	**98.00**	**98.00**
CV (%)	**3.00**	**10.80**	**6.90**	**7.00**
LSD (0.05)	**7.10**	**2.20**	**1.80**	**10.90**

4.3 Pairwise mean difference among genotypes

Pairwise mean differences among the genotypes ranged from 11.10 - 55.20 which was higher than the LSD value 10.94 **(Appendix- 2)** indicating significant variation found among the germplasm. The highest pairwise mean difference according to LSD value was observed in 'Salta begun and wild species (*S. torvum*)' (55.20) pair and it was followed by 'Salta Begun and wild species *Solanum sisymbriifolium'* (54.75), Nilphamari local and wild species *Solanum torvum* (53.70). The lowest mean difference was observed between 'Ashary and Comilla local' and 'Lalmoni Local-2 and Comilla Local (11.10)' **(Appendix- 2).** The result indicated that the 25 local eggplant genotypes and wild species had clear phenotypic diversity and it could be a leading factor in the future experiment regarding crop improvement program.

4.4 Correlation Analysis

Correlation analysis was done for four yield contributing traits of the studied genotypes. Days to 50% flowering had a positive correlation with branch per plant. With the increased number of days to flowering generated increased number of branch per plant (+ 0.12). Days to 50% flowering expressed negative correlation with both the yield per plant and yield per plot (- 0.15), indicating the increase value of days to flowering decreases the yield of individual plants as well as plot. Yield per plant had a positive correlation with yield per plot (+1) (Table 9).

Table 8. Correlation between yield contributing traits of different genotypes (below diagonal)

Traits	Days to 50% flowering	No. of branch per plant	Yield per plant	Yield per plot
Days to 50% flowering	***			
No. of branch per plant	0.12	***		
Yield per plant	-0.15	-0.38	***	
Yield per plot	-0.15	-0.38	1	***

4.5 Clustering of eggplant genotypes and wild relatives based on Scattered diagram

The study of 25 local eggplant genotypes and two wild species revealed different clusters according to the phenotypic traits analysis based on scattered diagram (Table 9 and Figure 2).

Table 9. Distribution of 27 eggplant genotypes in 4 different clusters

Cluster	No. of genotype	Name of genotypes
A	6	Cricket, Iswardi, Khotkhotia, Kurigram local, Nilphamari local and Rangpur local-2
B	6	Comilla local, Jamalpur local, Jessore local-1, Lalmoni local-2, Rangpur local-3 and Thakurgaon local
C	10	Bogra local, Dohazari, Jessore local-2, Jessore local-3, Jessore local-4, Lalmoni local-1, Narsingdi local, Rangpur local-1, Sada Khulna and Dinajpur local
D	5	Ashary, Khulna local-1,Salta begun, wild species *Solanum sisymbriifolium* and *Solanum torvum*

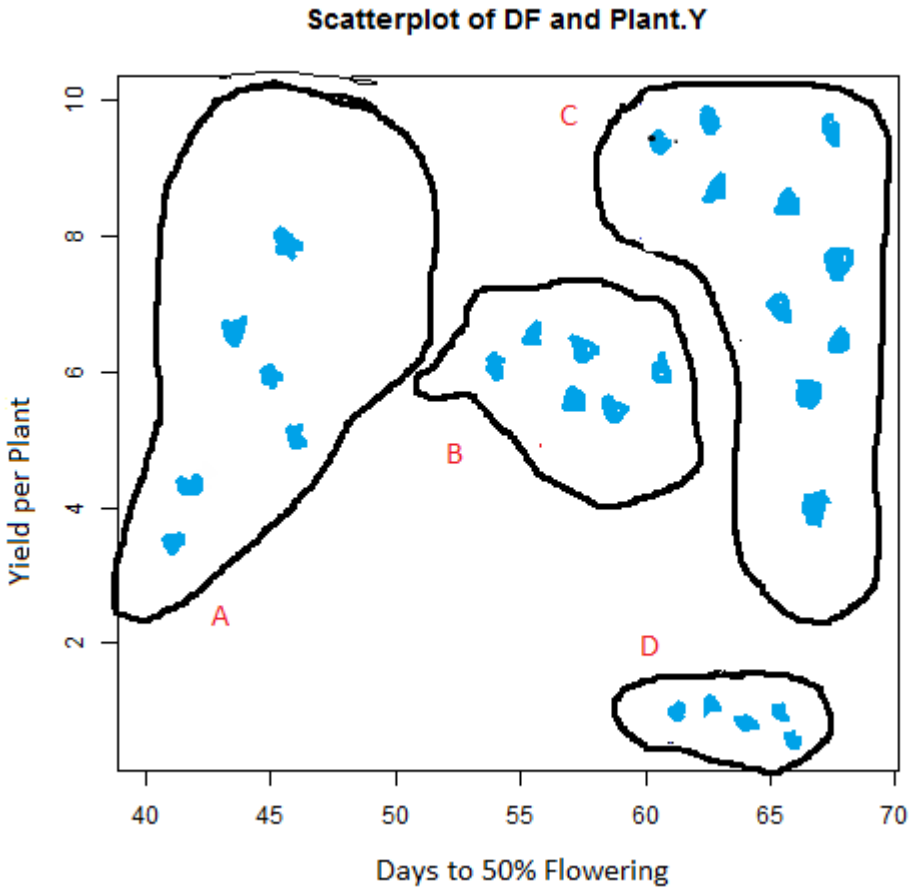

Figure 2. Clustering of twenty five local and two wild species of eggplant on the basis of scattered diagram.

4.6 Sub-experiment 2: Molecular diversity analysis of local eggplant germplasm and wild relatives

To evaluate more information about eggplant genetics, morphological analysis was not sufficient enough. Thats why molecular diversity was analyzed through DNA profiling. Highly polymorphic and repeatable PCR based microsatellite markers or Simple Sequence Repeat (SSRs) markers were used here to assess the polymorphism, diversity and similarity within those local and wild relatives.

4.6.1 DNA extraction

The genomic DNA extraction of 25 local eggplant genotypes and 2 wild species were done by using the CTAB method with minor modification. RNA sharing was removed by applying RNAse treatment. Finally, the purified DNA was stored at -20^{o}C freezer for further use.

4.6.2 DNA confirmation and quantification

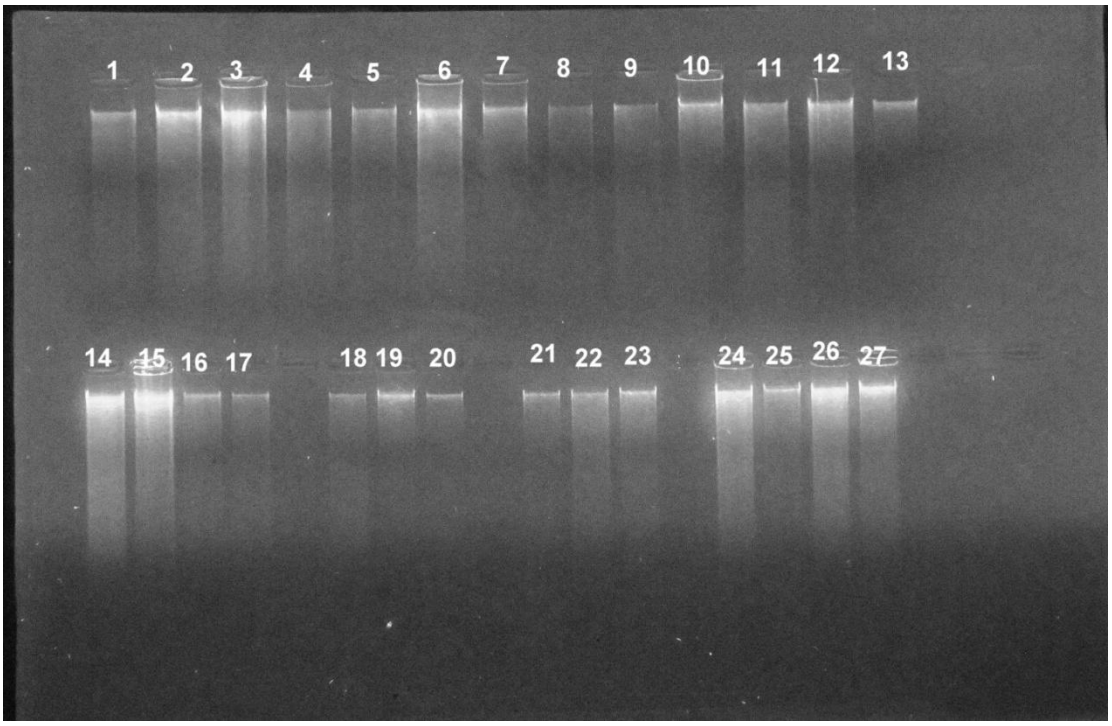

Plate 1. Confirmation of genomic DNA of 25 eggplant and two wild relatives in agarose gel.

(Lane 1: Salta begun; Lane 2: Ashary; Lane 3: Lalmoni local-1; Lane 4: Lalmoni local-2; Lane 5: Kurigram local; Lane 6: Khotkhotia; Lane 7: Cricket; Lane-8: Rangpur local-1; Lane 9: Rangpur local-2; Lane 10: Rangpur local-3; Lane11: Nilphamari local; Lane 12: Dinajpur local ; Lane 13: Thakurgaon local; Lane 14: Bogra local;Lane 15: Iswardi local; Lane 16: Jessore local-1; Lane 17: Jessore local-2;Lane 18: Jessore local-3;Lane 19: Jessore local-4;Lane 20: Sada khulna;lane 21: Khulna local-1; Lane 22: Jamalpur local; Lane 23: Narsingdi local; Lane 24: Comilla Local;Lane 25: Dohazari; Lane 26: Wild species *Solanum sisymbriifolium*; Lane 27: Wild species *Solanum torvum.*

4.6.3 Microsatellite primers or SSR markers for PCR amplification

Five SSR primers viz. EM114, EM104, smSSR01, smSSR04, EP82 were selected for PCR amplification of eggplant germplasm. These primers are highly specific for amplification of DNA and reproducible in nature.

4.6.4 Primer test

Primer test was conducted to verify the reproducibility of selected primers with randomly selected genomic DNA of eggplant germplasm. It was observed that all two SSR primer gave very clean and prominent DNA band in all the random sample of eggplant. DNA amplification was done by two SSR primers viz. EM 114 and EPSSR 82. Both the primer were successfully performed. The DNA amplification pattern is given in the Plate 2 and 3.

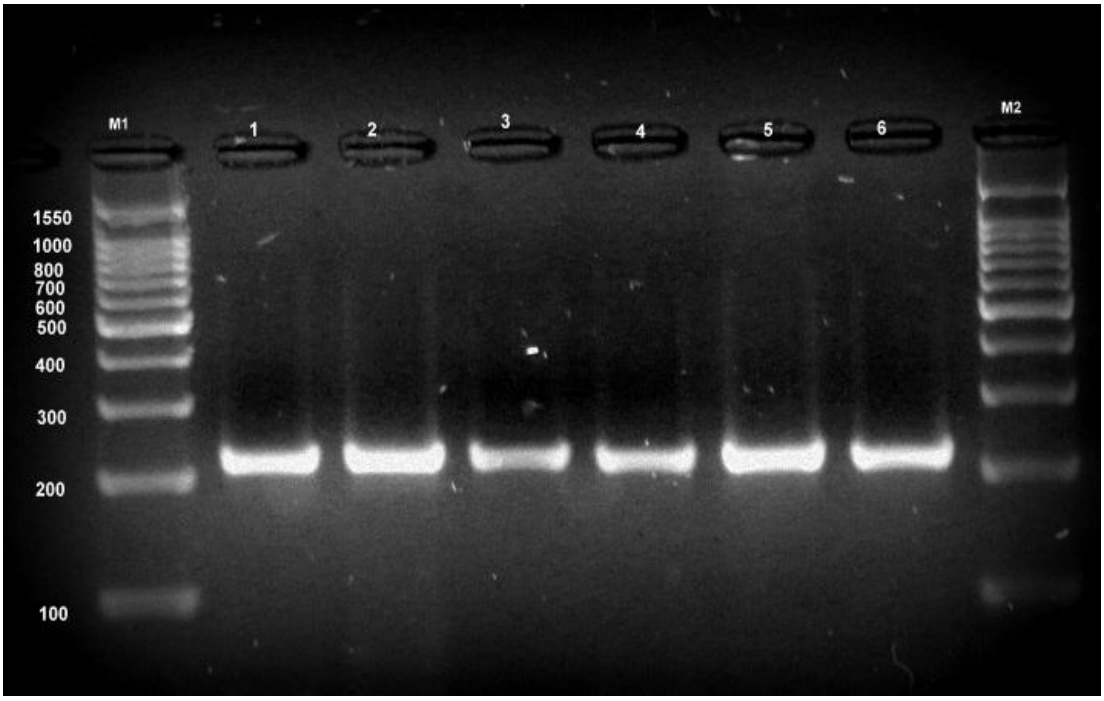

Plate 2. Primer test: DNA amplification by EM114 primer with randomly selected five eggplant genotypes.M1 and M2 is 100bp DNA ladder (Promega)

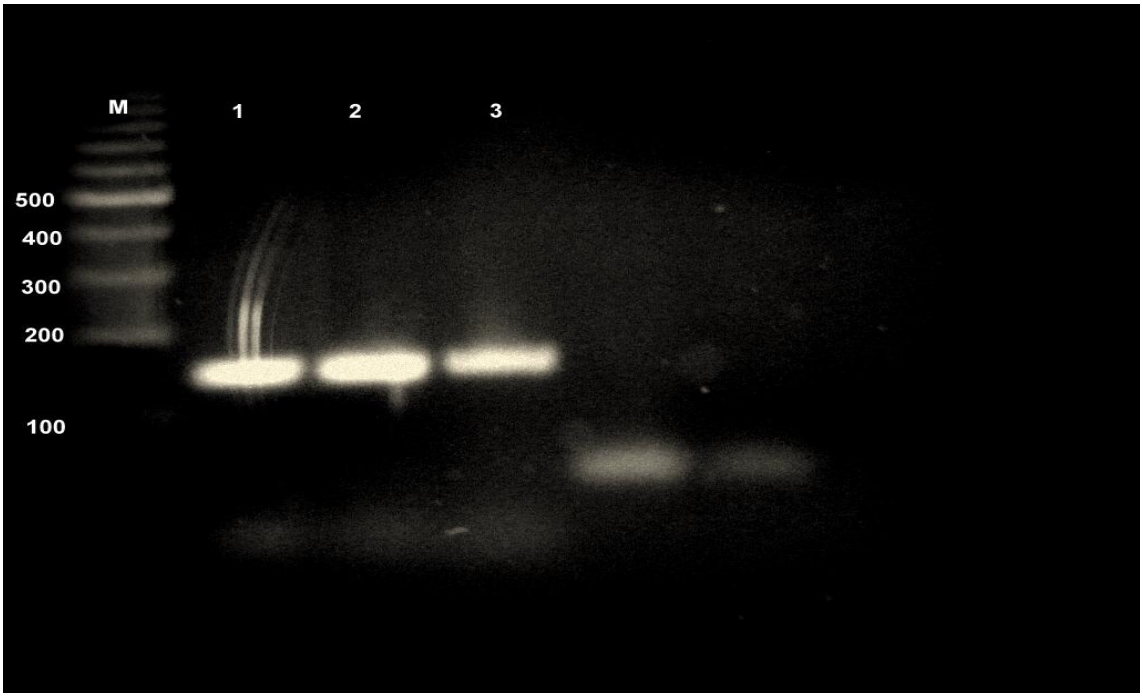

Plate 3 Primer test: DNA amplification by EPSSR82 primer with randomly selected three eggplant genotypes. M is 100bp DNA ladder (Promega).

4.7 DNA amplification by SSR markers and its polymorphism

Five SSR primers *viz*., EM114, EM120, smSSR01, amSSR04 and EPSSR82 produced different banding pattern separately with 25 eggplant genotypes and two wild relative. The amplifications of each SSR primers are presented in Table 10 and Plate 4 to 8.

The SSR primer EM114 produced only one DNA fragment among all the genotypes under study. The approximate fragment size was 225 bp. It was a monomorphic DNA band which was common in all the genotypes. The amplification product is presented in Plate 4.

Two fragments of DNA amplification were noticed by the SSR primer EM120. The size of amplification ranged from 50 to 180 bp. All the genotypes produced 180 bp fragment which indicated a monomorphic band. Whereas, the genotypes Salta begun, Ashary, Lalmoni local-1, Cricket, Nilphamari local and Dinajpur local were able to produce 80 bp polymorphic bands.

The SSR primer smSSR01 was able to amplify three fragments of DNA among all the individuals. The DNA product ranged from 200 to 400 bp. Among them 300 bp fragment was common in all genotypes. The germplasm Khotkhotia, Thakurgaon local, Bogra local and Khulna local-1 showed second amplification of DNA band. It's indicated that the second fragment at 320 bp is polymorphic in nature. Kurigram local, wild species *Solanum sisymbriifolium* and *Solanum torvum* produced third amplification at 250 bp, which was polymorphic.

The SSR primer EPSSR82 has the ability to amplify three fragment of DNA among all the experimental materials. The band size ranged from 50 to 180 bp. It was noticed that 180 bp fragment was common in all the genotypes and was monomorphic for all. The genotypes Salta begun, Ashary, Lalmoni local-1, Kurigram local, Cricket, Rangpur local, Thakurgaon local, Bogra local, Iswardi and Jessore local-3 were able to regenerate two additional DNA bands between the size ranging from 50 to 70 bp. The above finding indicated that, two polymorphic DNA were regenerated by the primer EPSSR82. A 50 bp DNA fragment was amplified by the primer smSSR04 and it was monomorphic for all the genotypes under study. On an average, five SSR primers were able to generate some total of 10 DNA amplification (10 band) with an average amplification for each primer was 2.0. Out of them, five were polymorphic among the genotypes under study and average polymorphism scored 1.0.

Khorsheduzzaman *et al*. (2008), found 22 amplified products using nine SSR primers with an average amplification for each primer 2.2. In their experiment, they studied over six eggplant genotypes and found that 70% were monomorphic and 30% were polymorphic for each of 9 primers.

Table 10. DNA amplification data regarding fragment size and number of polymorphic band with 27 genotypes

Primer no.	Primers' Name	Primer sequences (5'-3')	(G+C) %	No.of DNA band(s)	No.of polymorphic band(s)	Band size ranges (bp)
1	EM114	For. AGCCTAAACTTGGTTGGTTTTTGC Rev.GAAGCTTTAAGAGCCTTCTATGCAG	43	1	0	225
2	EM120	For. GGATCAACTGAAGAGCTGGTGGTT Rev.CAGAGCTTCAATGTTCCATTTCACA	44	2	1	50-180
3	EPSSR82	For. ACATGCCACTCATGTTGGTG Rev. CTTCAGCCATGGACCACATT	50	3	2	50-180
4	smSSR01	For. GTGACTACGGTTTCACTGGT Rev. GATGACGACGACGATAATAGA	46	3	2	200 - 400
5	smSSR04	For. AATGAGTCAGAAACCACGCC Rev. CGTTTAACCTTTGGCTCGGAA	49	1	0	50-80
Total	-	-	-	10	5	-
Mean	-	-	-	2.0	1.0	-

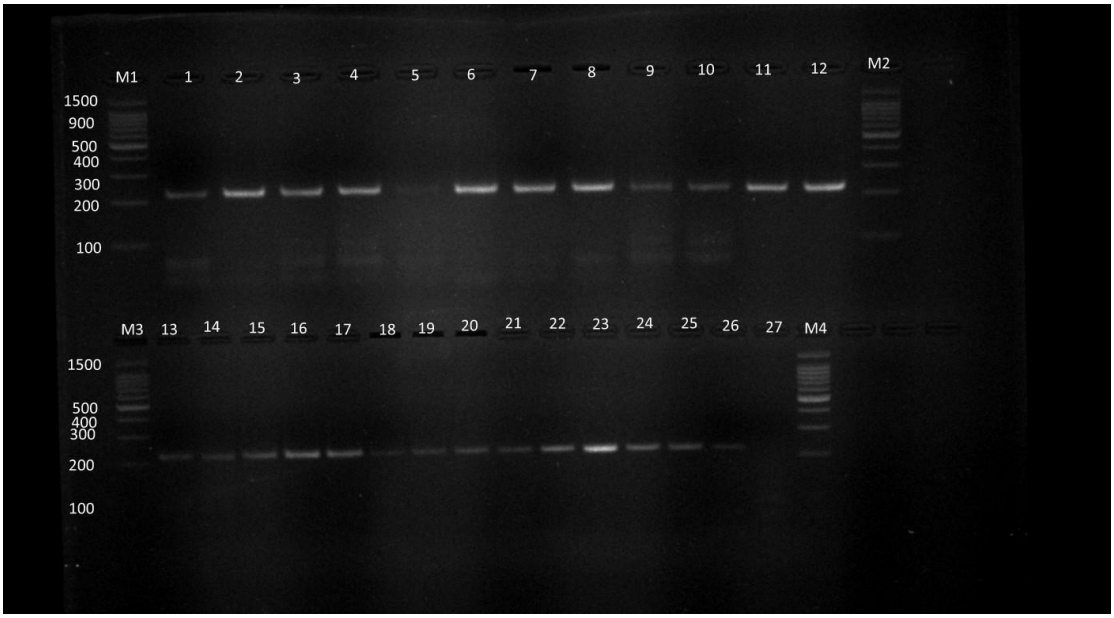

Plate 4. SSR profile of 27 local and wild eggplant germplasm using primer EM114.

(Lane 1: Salta begun; Lane 2: Ashary; Lane 3: Lalmoni local-1; Lane 4: Lalmoni local-2; Lane 5: Kurigram local; Lane 6: Khotkhotia; Lane 7: Cricket; Lane-8: Rangpur local-1; Lane 9: Rangpur local-2; Lane 10: Rangpur local-3; Lane11: Nilphamari local; Lane 12: Dinajpur local ; Lane 13: Thakurgaon local; Lane 14: Bogra local; Lane 15: Iswardi local; Lane 16: Jessore local-1; Lane 17: Jessore local-2; Lane 18: Jessore local-3; Lane 19: Jessore local-4; Lane 20: Sada khulna; lane 21: Khulna local-1; Lane 22: Jamalpur local; Lane 23: Narsingdi local; Lane 24:Comilla Local; Lane 25: Dohazari; Lane 26 : Wild species *Solanum sisymbriifolium*; Lane 27: Wild species *Solanum torvum* and M1=M2=M3=M4=100 bp DNA ladder).

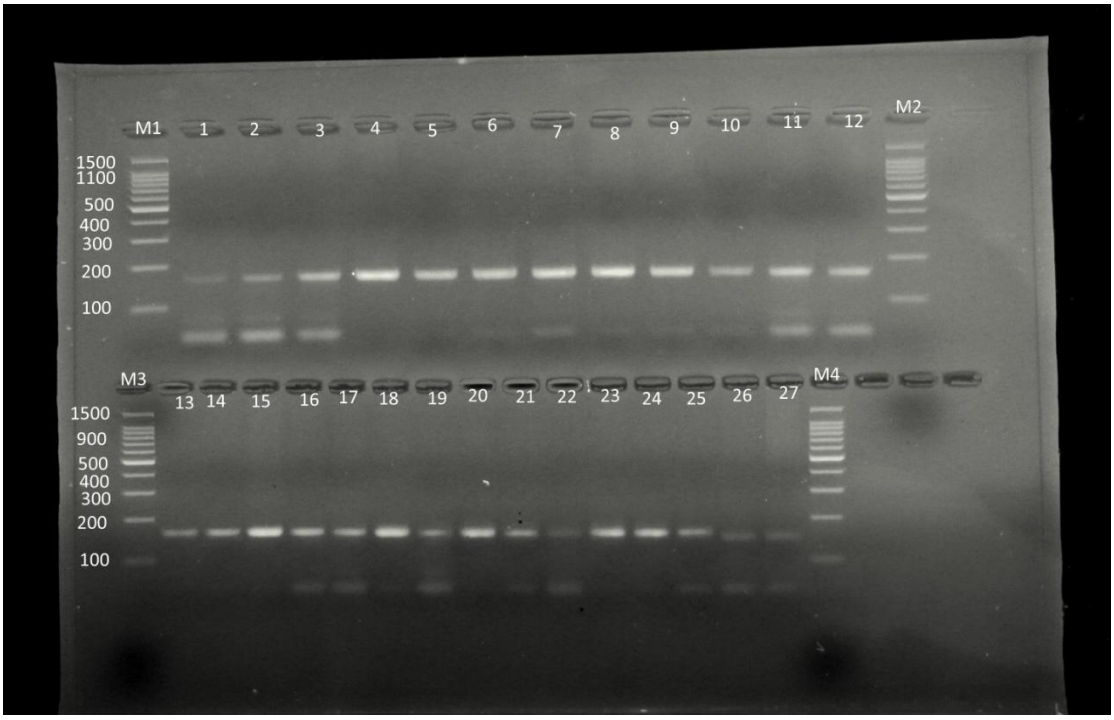

Plate 5. SSR profile of 27 local and wild eggplant germplasm using primer EM120.

(Lane 1: Salta begun; Lane 2: Ashary; Lane 3: Lalmoni local-1; Lane 4: Lalmoni local-2; Lane 5: Kurigram local; Lane 6: Khotkhotia; Lane 7: Cricket; Lane-8: Rangpur local-1; Lane 9: Rangpur local-2; Lane 10: Rangpur local-3; Lane11: Nilphamari local; Lane 12: Dinajpur local ; Lane 13: Thakurgaon local; Lane 14: Bogra local; Lane 15: Iswardi local; Lane 16: Jessore local-1; Lane 17: Jessore local-2; Lane 18: Jessore local-3; Lane 19: Jessore local-4; Lane 20: Sada khulna; lane 21: Khulna local-1; Lane 22: Jamalpur local; Lane 23: Narsingdi local; Lane 24:Comilla Local; Lane 25: Dohazari; Lane 26 : Wild species *Solanum sisymbriifolium*; Lane 27: Wild species *Solanum torvum* and M1=M2=M3=M4=100 bp DNA ladder).

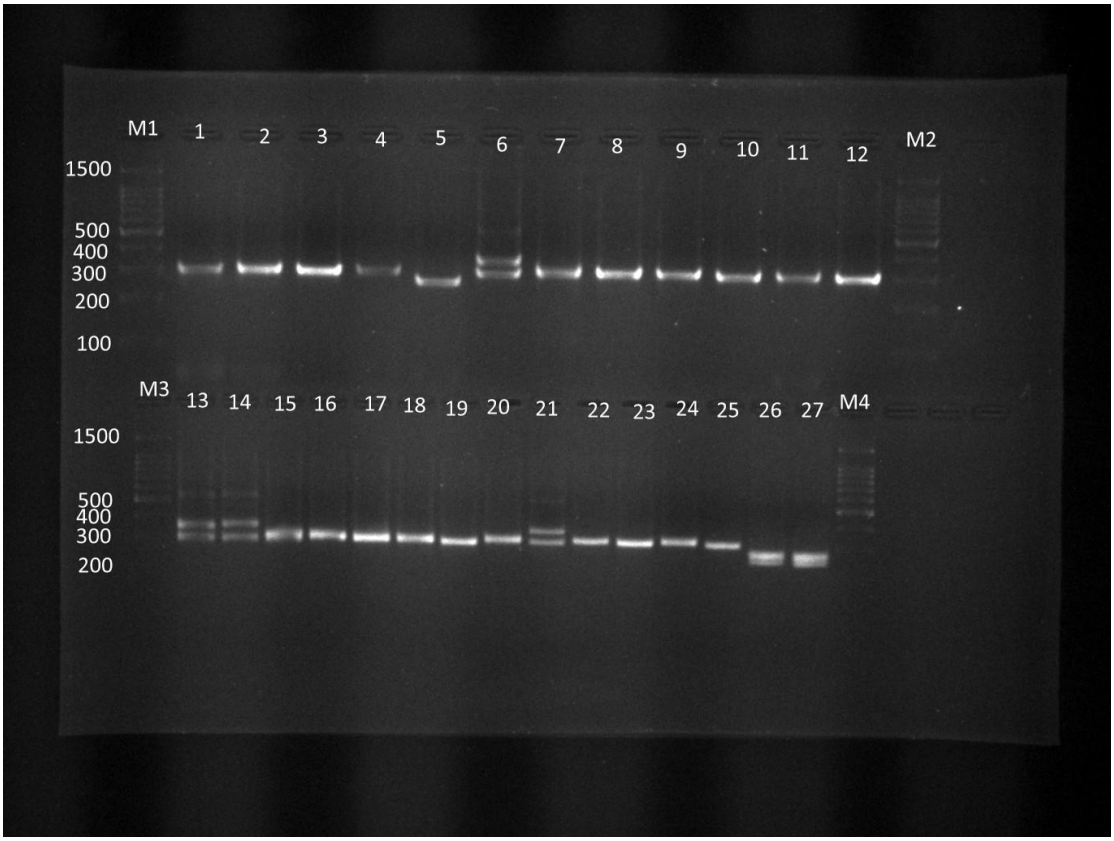

Plate 6. SSR profile of 27 local and wild eggplant germplasm using primer smSSR01.

(Lane 1: Salta begun; Lane 2: Ashary; Lane 3: Lalmoni local-1; Lane 4: Lalmoni local-2; Lane 5: Kurigram local; Lane 6: Khotkhotia; Lane 7: Cricket; Lane-8: Rangpur local-1; Lane 9: Rangpur local-2; Lane 10: Rangpur local-3; Lane11: Nilphamari local; Lane 12: Dinajpur local ; Lane 13: Thakurgaon local; Lane 14: Bogra local; Lane 15: Iswardi local; Lane 16: Jessore local-1; Lane 17: Jessore local-2; Lane 18: Jessore local-3; Lane 19: Jessore local-4; Lane 20: Sada khulna; lane 21: Khulna local-1; Lane 22: Jamalpur local; Lane 23: Narsingdi local; Lane 24:Comilla Local; Lane 25: Dohazari; Lane 26 : Wild species *Solanum sisymbriifolium*; Lane 27: Wild species *Solanum torvum* and M1=M2=M3=M4=100 bp DNA ladder).

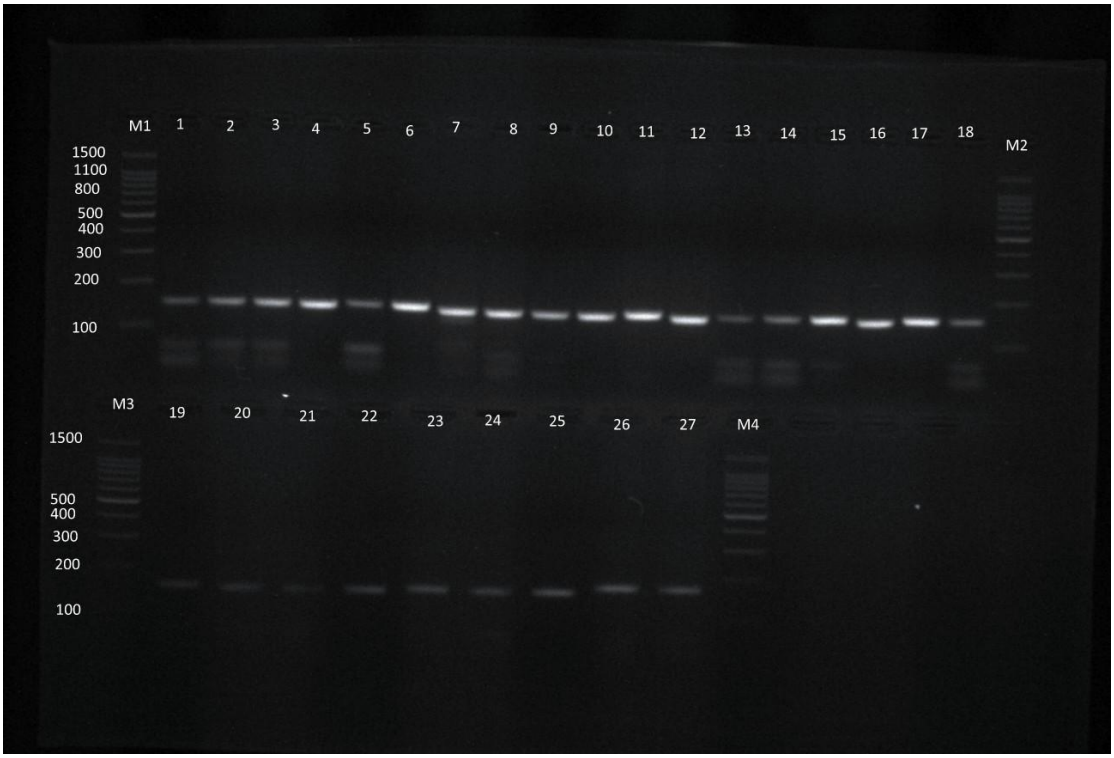

Plate 7. SSR profile of 27 local and wild eggplant germplasm using primer EPSSR82.

(Lane 1: Salta begun; Lane 2: Ashary; Lane 3: Lalmoni local-1; Lane 4: Lalmoni local-2; Lane 5: Kurigram local; Lane 6: Khotkhotia; Lane 7: Cricket; Lane-8: Rangpur local-1; Lane 9: Rangpur local-2; Lane 10: Rangpur local-3; Lane11: Nilphamari local; Lane 12: Dinajpur local ; Lane 13: Thakurgaon local; Lane 14: Bogra local; Lane 15: Iswardi local; Lane 16: Jessore local-1; Lane 17: Jessore local-2; Lane 18: Jessore local-3; Lane 19: Jessore local-4; Lane 20: Sada khulna; lane 21: Khulna local-1; Lane 22: Jamalpur local; Lane 23: Narsingdi local; Lane 24:Comilla Local; Lane 25: Dohazari; Lane 26 : Wild species *Solanum sisymbriifolium*; Lane 27: Wild species *Solanum torvum* and M1=M2=M3=M4=100 bp DNA ladder).

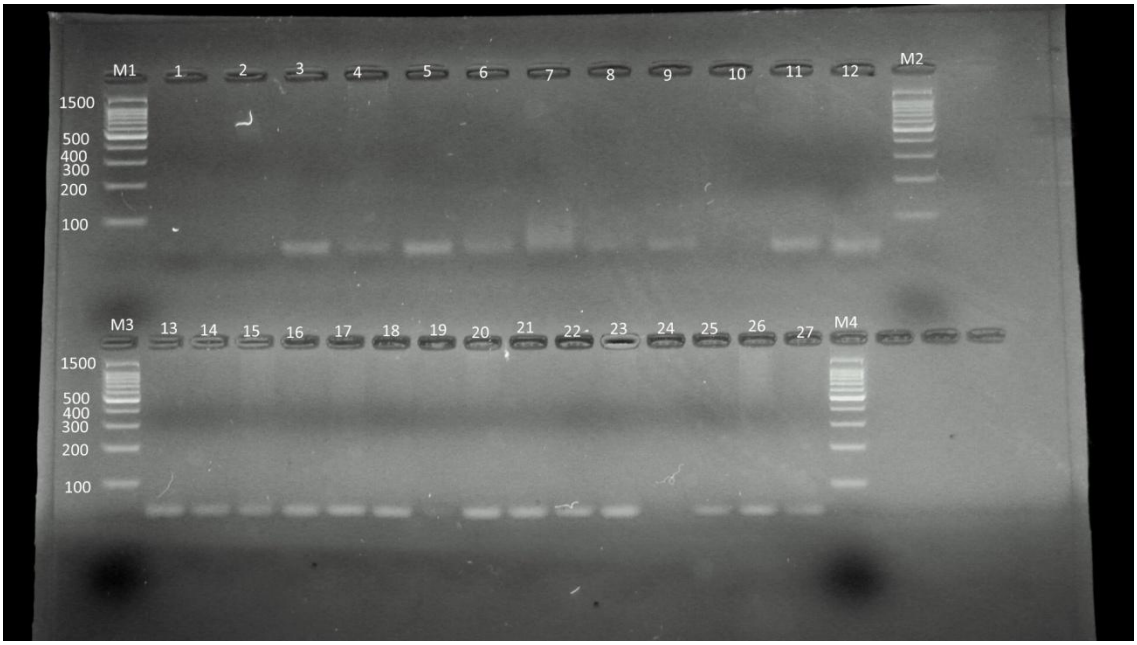

Plate 8: SSR profile of 27 local and wild eggplant germplasm using primer smSSR04.

(Lane 1: Salta begun; Lane 2: Ashary; Lane 3: Lalmoni local-1; Lane 4: Lalmoni local-2; Lane 5: Kurigram local; Lane 6: Khotkhotia; Lane 7: Cricket; Lane-8: Rangpur local-1; Lane 9: Rangpur local-2; Lane 10: Rangpur local-3; Lane11: Nilphamari local; Lane 12: Dinajpur local ; Lane 13: Thakurgaon local; Lane 14: Bogra local; Lane 15: Iswardi local; Lane 16: Jessore local-1; Lane 17: Jessore local-2; Lane 18: Jessore local-3; Lane 19: Jessore local-4; Lane 20: Sada khulna; lane 21: Khulna local-1; Lane 22: Jamalpur local; Lane 23: Narsingdi local; Lane 24:Comilla Local; Lane 25: Dohazari; Lane 26 : Wild species *Solanum sisymbriifolium*; Lane 27: Wild species *Solanum torvum* and M1=M2=M3=M4=100 bp DNA ladder).

4.8 Allelic frequency, gene diversity and Polymorphism Information Content (PIC)

Allelic frequency, gene diversity and Polymorphism Information Content (PIC) value of experimental genotypes are presented in Table 11. PCR products of five SSR markers were characterized. A total 10 alleles were detected for the five polymorphic SSR loci, with an average number of alleles/locus of 2.0 and a range between 2 (EM120) and 3 (EPSSR82, smSSR01) (Table 11). The frequency of the major allele ranged between 0.33 (EPSSR 82) to 0.56 (EM120, smSSR01) with an average value of 0.49. Polymorphic Information Content (PIC) value for the 5 markers ranged from 0.37 (smSSR01) to 0.67 (EPSSR 82) and the average PIC value was 0.54. Gene diversity ranged between 0.49 (smSSR 01) to 0.72 (EPSSR 82) with an average of 0.61.

The highest PIC value (0.67) was obtained for EPSSR82 (Table 11) followed by EM114 (0.57), EM120 (0.55) and smSSR04 (0.53) respectively. The lowest PIC value 0.37 was obtained for smSSR01 (Table 11). PIC value revealed that EPSSR82 was considered as the best marker for 27 eggplant germplasm followed by EM114 and EM120, respectively. smSSR04 could be considered as the least powerful marker.

The results indicate that the 25 local eggplant landraces present a high degree of homozygosity and are closely related to the wild variety *Solanum torvum* and *Solanum sysimbriifolium*, and also considerable intra-varietal group diversity, and a certain degree of genetic differentiation and polymorphism really do exist.

Vilanova *et al.* (2014) used 19 genomic SSRs for the molecular characterization of 30 eggplant accessions corresponding to the four cultivar groups. The polymorphism information content (PIC) of SSR markers ranged from 0.07 to 0.77, with an average value of PIC=0.50. The mean observed heterozygosity (Ho) presented a very low value Ho=0.01, while the mean expected heterozygosity (He) had a value of He=0.57. Genomic SSRs that previously proved to be highly polymorphic in eggplant (Vilanova *et al.*, 2012) have been found to be of great value for evaluating the genetic diversity and relationships in a collection of eggplants from different cultivar groups.

Table 11. Major allelic frequency, gene diversity and PIC value of different genotypes

Markers	Obs. No.	Availability	Allele no.	Major allele frequency	Gene diversity	PIC value
EM114	27	1.00	1.0	0.52	0.63	0.57
EM120	27	1.00	2.0	0.56	0.61	0.55
EPSSR 82	27	1.00	3.0	0.33	0.72	0.67
smSSR 01	27	1.00	3.0	0.56	0.49	0.37
smSSR 04	27	1.00	1.0	0.48	0.61	0.53
Mean	**27**	**1.00**	**2.0**	**0.49**	**0.61**	**0.54**

4.9 Nei's Genetic Distance and Genetic Identity

The value of pair-wise comparisons of Nei's (1972) genetic distance (D) among 25 local and two wild relatives of eggplant were computed from combined data for the 5 primers, ranged from 0.200 to 1.000 with an average of 0.600.

Comparatively higher genetic distance (1.000) was observed between a number of genotypes. Among them Ashary showed highest genetic dissimilarity with maximum number (14) of genotypes viz., Bogra local, Comilla local, Dohazari, Jamalpur local, Jessore local-1, Jessore local-2, Jessore local-3, Jessore local-4, Khulna local, Narsingdi local, Sada Khulna, Thakurgaon local, 2 wild species (*Solanum sisymbrifolium* and *Solanum torvum*). The wild species *Solanum torvum* showed highest genetic distance among 12 eggplant genotypes (Table12: below diagonal).

The highest genetic distance between them indicated that genetically they are diversed. This value indicated the genetic dissimilarity among them. Genotypes pair

with higher(1.000) of genetic distance is more dissimilar than a pair with a lower value. The lowest genetic distance (0.200) was found in a variety of pairs indicating that they are genetically much closer among them. The average genetic distance among the 27 genotypes was quantified as 0.600.

Nei's genetic identity between 27 genotypes were calculated from the combined data of 5 primers and the value ranged from 0.200 to 1.000. The highest Nei's genetic identity was observed in various genotype pairs. Among them Bogra local showed maximum genetic similarities with maximum number (10) of genotypes viz., Iswardi, Jamalpur local, Jessore local-1, Jessore local-2, Jessore local-4, Khulna local-1, Narsingdi local, Sada Khulna, Thakurgaon local. Whereas, the lowest (0.200) value was also frequently observed in a number of pairs (Table 12. above diagonal). From Nei's genetic distance and identity value it was clearly revealed that the 25 eggplant genotypes and 2 wild species had distinct genetic diversity.

Table 12. Summary of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) values between 25 local genotypes and 2 wild species of eggplant.

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	**	0.0	0.0	0.6	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.8	1.0	0.6	0.2	0.0	0.6	0.6	0.4	0.0	1.0	0.0	0.0	0.0
2	1.0	**	0.6	0.0	0.2	0.6	0.8	0.8	0.8	0.8	1.0	0.8	0.0	0.8	0.0	0.0	0.0	0.2	0.8	0.0	0.0	0.0	0.8	0.0	1.0	0.4	0.6
3	1.0	0.4	**	0.0	0.0	1.0	0.6	0.8	0.6	0.6	0.6	0.8	0.0	0.8	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.8	0.0	0.6	0.8	1.0
4	0.4	1.0	1.0	**	0.4	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.8	0.6	0.6	0.4	0.0	1.0	1.0	0.8	0.0	0.6	0.0	0.0	0.0
5	0.8	0.8	1.0	0.6	**	0.0	0.2	0.2	0.4	0.4	0.2	0.2	0.4	0.2	0.2	0.2	0.4	1.0	0.2	0.4	0.4	0.6	0.2	0.2	0.2	0.0	0.0
6	1.0	0.4	0.0	1.0	1.0	**	0.4	0.8	0.6	0.6	0.6	0.8	0.0	0.8	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.8	0.0	0.6	0.8	1.0
7	0.8	0.2	0.4	0.8	0.8	0.4	**	0.8	0.8	0.8	0.8	0.8	0.0	0.8	0.2	0.2	0.0	0.2	0.8	0.2	0.2	0.0	0.8	0.2	0.8	0.4	0.6
8	1.0	0.2	0.2	1.0	0.8	0.2	0.2	**	0.8	0.8	0.8	1.0	0.0	1.0	0.0	0.0	0.0	0.2	1.0	0.0	0.0	0.0	1.0	0.0	0.8	0.6	0.8
9	1.0	0.2	0.4	1.0	0.6	0.4	0.2	0.2	**	1.0	0.8	0.8	0.2	0.8	0.0	0.0	0.2	0.4	0.8	0.0	0.0	0.2	0.8	0.0	0.8	0.4	0.6
10	1.0	0.2	0.4	1.0	0.6	0.4	0.2	0.2	0.0	**	0.8	0.8	0.2	0.8	0.0	0.0	0.2	0.4	0.8	0.0	0.0	0.2	0.8	0.0	0.8	0.4	0.6
11	1.0	0.0	0.4	1.0	0.8	0.4	0.2	0.2	0.2	0.2	**	0.8	0.0	0.8	0.0	0.0	0.0	0.2	0.8	0.0	0.0	0.0	0.8	0.0	1.0	0.4	0.6
12	1.0	0.2	0.2	1.0	0.8	0.2	0.2	0.0	0.2	0.2	0.2	**	0.0	1.0	0.0	0.0	0.0	0.2	1.0	0.0	0.0	0.0	1.0	0.0	0.8	0.6	0.8
13	0.4	1.0	1.0	0.4	0.6	1.0	1.0	1.0	0.8	0.8	1.0	1.0	**	0.0	0.8	0.6	1.0	0.4	0.0	0.6	0.6	0.8	0.0	0.6	0.0	0.0	0.0

Table 12. (Cont'd) Summary of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) values between 25 local genotypes and 2 wild species of eggplant

14	1.0	0.2	0.2	1.0	0.8	0.2	0.2	0.0	0.2	0.2	0.2	0.0	1.0	**	0.0	0.0	0.0	0.2	1.0	0.0	0.0	0.0	1.0	0.0	0.8	0.6	0.8
15	0.2	1.0	1.0	0.2	0.8	1.0	0.8	1.0	1.0	1.0	1.0	1.0	0.2	1.0	**	0.8	0.8	0.2	0.0	0.8	0.8	0.6	0.0	0.8	0.0	0.0	0.0
16	0.0	1.0	1.0	0.4	0.8	1.0	0.8	1.0	1.0	1.0	1.0	1.0	0.4	1.0	0.2	**	0.6	0.2	0.0	0.6	0.6	0.4	0.0	1.0	0.0	0.0	0.0
17	0.4	1.0	1.0	0.4	0.6	1.0	1.0	1.0	0.8	0.8	1.0	1.0	0.0	1.0	0.2	0.4	**	0.4	0.0	0.6	0.6	0.8	0.0	0.6	0.0	0.0	0.0
18	0.8	0.8	1.0	0.6	0.0	1.0	0.8	0.8	0.6	0.6	0.8	0.8	0.6	0.8	0.8	0.8	0.6	**	0.2	0.4	0.4	0.6	0.2	0.2	0.2	0.0	0.0
19	1.0	0.2	0.2	1.0	0.8	0.2	0.2	0.0	0.2	0.2	0.2	0.0	1.0	0.0	1.0	1.0	1.0	0.8	**	0.0	0.0	0.0	1.0	0.0	0.8	0.6	0.8
20	0.4	1.0	1.0	0.0	0.6	1.0	0.8	1.0	1.0	1.0	1.0	1.0	0.4	1.0	0.2	0.4	0.4	0.6	1.0	**	1.0	0.8	0.0	0.6	0.0	0.0	0.0
21	0.4	1.0	1.0	0.0	0.6	1.0	0.8	1.0	1.0	1.0	1.0	1.0	0.4	1.0	0.2	0.4	0.4	0.6	1.0	0.0	**	0.8	0.0	0.6	0.0	0.0	0.0
22	0.6	1.0	1.0	0.2	0.4	1.0	1.0	1.0	0.8	0.8	1.0	1.0	0.2	1.0	0.4	0.6	0.2	0.4	1.0	0.2	0.2	**	0.0	0.4	0.0	0.0	0.0
23	1.0	0.2	0.2	1.0	0.8	0.2	0.2	0.0	0.2	0.2	0.2	0.0	1.0	0.0	1.0	1.0	1.0	0.8	0.0	1.0	1.0	1.0	**	0.0	0.8	0.6	0.8
24	0.0	1.0	1.0	0.4	0.8	1.0	0.8	1.0	1.0	1.0	1.0	1.0	0.4	1.0	0.2	0.0	0.4	0.8	1.0	0.4	0.4	0.6	1.0	**	0.0	0.0	0.0
25	1.0	0.0	0.4	1.0	0.8	0.4	0.2	0.2	0.2	0.2	0.0	0.2	1.0	0.2	1.0	1.0	1.0	0.8	0.2	1.0	1.0	1.0	0.2	1.0	**	0.4	0.6
26	1.0	0.6	0.2	1.0	1.0	0.2	0.6	0.4	0.6	0.8	0.6	0.4	1.0	0.4	1.0	1.0	1.0	1.0	0.4	1.0	1.0	1.0	0.4	1.0	0.6	**	0.8
27	1.0	0.4	0.0	1.0	1.0	0.0	0.4	0.2	0.4	0.4	0.4	0.2	1.0	0.2	1.0	1.0	1.0	1.0	0.2	1.0	1.0	1.0	0.2	1.0	0.4	0.2	**

1=Ashary , 2= Bogra Local ,3= Comilla Local ,4= Cricket,5= Dinajpur Local, 6= Dohazari, 7= Iswardi, 8= Jamalpur Local, 9= Jessore Local-1, 10= Jessore Local-2, 11= Jessore Local-3, 12= Jessore Local-4, 13= Khotkhotia, 14= Khulna Local-1 , 15= Kurigram Local , 16= Lalmoni Local-1 , 17= Lalmoni Local-2 , 18= Nilphamari local , 19= Narsingdi local , 20= Rangpur Local-1 , 21= Rangpur Local-2, 22= Rangpur Local-3 ,23 =Sada Khulna , 24= Salta Begun, 25= Thakurgaon local, 26= Wild species (*Solanum torvum)*, 27= Wild species (*Solanum sisymbriifolium*).

4.10 UPGMA dendrogram

A dendrogram was constructed based on the Nei's genetic distance calculated from 25 eggplant genotypes and two wild species. Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated the segregation of 27 genotypes into two main clusters I & II (Figure. 3).

Ashary, Khotkhotia, Kurigram local, Lalmoni local-1, Lalmoni local-2, Salta begun, Cricket ,Dinajpur local, Nilphamari local, Rangpur-1, Rangpur-2, Rangpur-3 formed cluster-I. On the other hand, Bogra local, Comilla local, Dohazari, Iswardi, Jamalpur local, Jessore local-1, Jessore local-2, Jessore local-3, Jessore local-4, Khulna local-1, Narsingdi local, Sada Khulna, Thakurgaon local, wild species (*Solanum sisymbriifolium*) and wild species (*Solanum torvum*) formed cluster-II.

In cluster-I, Ashary, Khotkhotia, Kurigram local, Lalmoni local-1, Lalmoni local-2, Salta begun formed sub-cluster I(a) and Cricket , Dinajpur local, Nilphamari local, Rangpur-1, Rangpur-2, Rangpur-3 formed sub-cluster-II(b).

In case of cluster-II, Bogra local, Comilla local, Dohazari, Iswardi, Jamalpur local, Jessore local-1, Jessore local-2, Jessore local-3, Jessore local-4, Khulna local-1, Narsingdi local, Sada Khulna, Thakurgaon local, wild species (*Solanum sisymbriifolium*) formed sub-cluster-II(a) and wild species (*Solanum torvum*) alone formed sub-cluster II(b).In this dendogram, eggplant genotypes of close genetic distance belong to same sub-cluster.The dendogram revealed that the genotypes that derivatives of genetically similar type clustered together.

Based on above result, it may be concluded that, the close relatives of the eggplant germplasm are grouped in the same cluster due to lower genetic distance and the genetically dissimilar germplasms were placed in another cluster due to higher genetic distance. It is clearly observed that wild species (*Solanum torvum*) is very much different from all the genotypes.

The result indicates that the low or high level genetic distance exits within the genotypes. A comparison is constructed on wild species (*Solanum torvum*) and with the other genotypes as Ashary, Khotkhotia, Kurigram local, Lalmoni local-1, Lalmoni local-2, Salta begun, Cricket , Dinajpur local , Nilphamari local, Rangpur-1, Rangpur-2, Rangpur-3 showed highest Nei's genetic distance (1.000) from wild species

(*Solanum torvum*) as they are released from different parental origin. On the otherhand, Wild-2 , Comilla local, Dohazari, Iswardi, Jamalpur local, Jessore local-1, Jessore local-2, Jessore local-3, Jessore local-4, Khulna local-1, Narsingdi local, Sada Khulna, Thakurgaon local showed lowest Nei's genetic distance as they belong to same parental origin.

As expected, *Solanum torvum* has the least similarity to the rest of the group. Although *S. torvum* belongs to the genus *Solanum*, but it does not belong to the two broadly categorized closely related eggplant wild relatives *S. incanum* and *S. melongena* (Knapp *et al*., 2013). While most of the eggplant wild species originated from Africa, *Solanum torvum* is native to West Indies, India, Myanmar, Thailand, Philippines, Malaysia, China and tropical America and widely naturalized in South and Southeast Asia (Nasir, 1985). It possesses a number of desirable horticultural traits such as disease resistance (Kumchai *et al*., 2013) and has medicinal uses (Yousaf *et al*., 2013). This result clearly indicates that different levels of genetic identity and distance present within the eggplant germplasm and shown in the UPMGA dendrogram (Figure 3).

Different levels of cluster analysis was reviewed which was performed by several scientists.

Caguiat *et al.* (2014) constructed a dendrogram with scale from 0.16 to 0.97 based on Jaccard's similarity coefficient. Separated the 32 accessions into 4 main clusters (*S. Melongena* and 3 small CWR clusters) and 8 sub-clusters (I-VIII) when a line was drawn at similarity coefficient of 0.42.

Valinova *et al*. (2014) designed an experiment with 19 SSR markers to analysis genetic diversity among 30 Spanish eggplant genotypes. A multivariate cluster analyses revealed that a considerable diversity exists within each of the cultivar groups. Listada de Gandía and Long cultivar groups were clearly separated from each other in different branches of phenogram. Overall, the results indicated that Spanish eggplant landraces present a high degree of homozygosis, considerable intra-cultivar group diversity, and a certain degree of genetic differentiation. This information is of interest for selection and breeding of eggplant as well as for germplasm conservation.

In Indian subcontinent eggplant germplasm found to be diverse. Germplasm from different regions shows a wide range of genetic diversity as well as phenotypic diversity indeed. This wide level of diversity among species of Solanum may be attributable to the fact that it is an ancient Old World plant (Whalen, 1979).

Based on the results shown above, it can be concluded that morphology-based analysis was effective in differentiating the eggplant accessions in the current germplasm collection in accordance with their known species and gene pool delineation. However, one must bear in mind that morphological traits are known to be largely influenced by the environment (Hillis, 1987) which could result in variation without associated changes at the DNA sequence level.

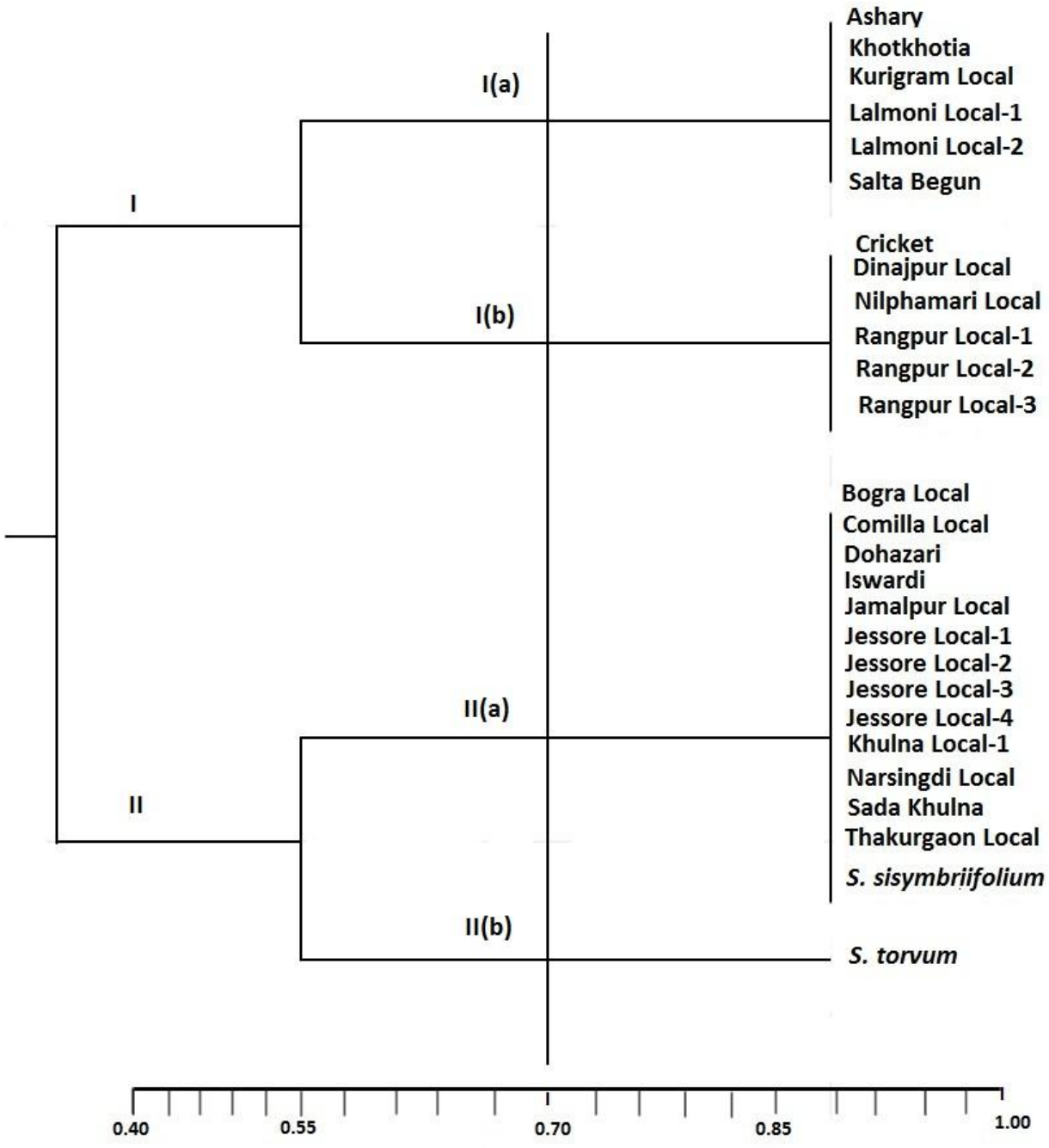

Figure 3. Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's (1972) genetic distance, summarizing data on differentiation for twenty-five local and two wild relatives of eggplant.

Chapter Five

SUMMARY AND CONCLUSION

Bangladesh has wide range of diverse eggplant landraces. This experiment was carried out to investigate the diversity and relatedness among twenty-five local and two wild species found in Bangladesh using five highly polymorphic Simple Sequence Repeats (SSR) markers. Morphological characterization of eggplant and its wild relatives were also investigated. Experiment was carried out in the field and Biotechnology Laboratory of Sher-e-Bangla Agricultural University, Dhaka.

Morphological features of each eggplant genotypes were clearly differentiated from the others. Three types of color variations *viz.* purple, pink and green were observed in the stem, leaf vein and fruit of the genotypes. Eggplant genotypes showed tremendous variation in fruit shape also. Among the genotypes, four types of shape of fruit were found such as long, oval, oblong and round shape. The highest individual fruit weight was observed in Iswardi (411g) followed by Sada Khulna (347g), Khotkhotia (345g) and the lowest was in wild species *Solanum sisymbriifolium* (10g). The highest yield per plant was observed in Salta Begun (9.8 kg) and the lowest observed in the wild species (0.6 kg). Analysis of variance showed significant differences among the genotypes for all the characters. Hence, there is an opportunity to select desirable germplasm with desired traits.

The highest pairwise mean difference according to LSD value was observed in 'Salta begun and *Solanum torvum* and it was 55.20 followed by 'Salta Begun and *Solanum sisymbriifolium*' (54.75), Nilphamari local and *Solanum torvum* (53.70) and the lowest mean difference was observed in both 'Ashary and Comilla local', 'Lalmoni local-2 and Comilla Local' and it was 11.10. From scattered diagram four clusters generated which differentiating 25 local and two wild relatives of eggplant. Each of the cluster containing the genotypes of similar characters. But, the cluster D had only five genotypes such as Ashary, Khulna local-1, Salta begun and two wild species *Solanum sisymbriifolium* and *Solanum torvum* indicating close relatedness among them could be used for future improvement.

Total ten DNA bands were generated from the five SSR primers *viz*. EM114, EM120, EPSSR82, smSSR01 and smSSR04. Amplified alleles ranged from 1 to 3 per locus with an average 2.0 alleles/locus were detected. The frequency of major allele ranged from 0.33 (EPSSR82) to 0.56 (EM120 and smSSR01) with an average value of 0.49. Among them three primers *viz.* EM120, EPSSR82 and smSSR01 were able to produce total five polymorphic bands with an average 1.0 for all the genotypes. SSR primer EPSSR82 and smSSR04 produced two polymorphic bands whereas, primer EM120 produced single polymorphic band. But, rest of two SSR primers such as EM114 and smSSR04 were not able to generate any polymorphic band. The Polymorphism Information Content (PIC) for all the markers ranged from 0.37 to 0.67 with an average value of PIC = 0.54. Gene diversity ranges from 0.49 to 0.72, with an average value of 0.61. SSR markers showed an average gene diversity of 0.61 for all the genotypes.

Nei's (1972) genetic distance and identity ranged from 0.200 to 1.000 were computed from the combined data of five primers. The highest genetic distance (1.000) observed between a number of genotypes among them Ashary showed maximum dissimilarities with 14 genotypes and wild species *Solanum torvum* showed dissimilarities with 12 genotypes. Dendrogram based on Nei's (1972) genetic distance using Unweighted Pair Group Method of Arithmetic Mean (UPMGA) segregated the 25 local and two wild relatives of eggplant into two major clusters. The first major cluster had twelve eggplant genotypes such as Ashary, Khotkhotia, Kurigram local, Lalmoni local-1, Lalmoni local-2, Salta begun, Cricket, Dinajpur local, Nilphamari local, Rangpur-1, Rangpur-2 and Rangpur-3. The second major cluster had rest of the fifteen genotypes together with two wild species. But, in case of cluster II, wild species *Solanum torvum* along fromed sub-cluster II(b) which differentiated it's diversity from all others genotypes.

It is concluded that SSR markers have been proved to be a powerful tool for molecular genetic analysis of eggplant germplasm for plant breeding programs to assess genetic diversity for the development of improved cultivars that would be able to survive from biotic and abiotic stresses. The results of this study can be used as a

baseline for the future diversity assessment and genetic analysis of eggplant landraces and wild relatives of Bangladesh.

The present work was carried out to assess genetic variation of local eggplant germplasm and wild relatives. However, a larger number of genotypes and a higher number of primers would be necessary to construct an appropriate relationship and diversity. Nevertheless, the current study is widely acceptable in all concerns. The present study might be used as a guideline for further research and the following points might be considered for sustaining the genetic qualities of eggplant in Bangladesh.

i. To obtain more precise result a large number of SSR markers should be used.

ii. A large number of germplasm should be included.

iii. High throughput molecular markers such as single nucleotide polymorphism (SNP) should be used for genome-wide converge of eggplant germplasm.

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APPENDICES

Appendix I. Analysis of Variance (ANOVA) for four yield contributing traits such as (a) Days of 50% Flowering; (b) Branch per Plant; (c) Yield per Plant and (d) Yield per Plot computed on the genotypes

I(a): ANOVA for the response variable Days to 50% Flowering

Item(s)	DF	Sum of Square	Mean Square	F value	P value
Replication	1	8.96	8.96	2.95	0.10
Genotype	26	4227.33	162.59	53.49	0.00
Error	26	79.04	3.04		

I(b) : ANOVA for the response variable Branch per Plant

Item(s)	DF	Sum of Square	Mean Square	F value	P value
Replication	1	0.02	0.02	0.06	0.08
Genotype	26	75.33	2.90	10.07	0.00
Error	26	7.48	0.29		

I(c) : ANOVA for the response variable Yield per Plant

Item(s)	DF	Sum of Square	Mean Square	F value	P value
Replication	1	0.07	0.07	0.36	0.55
Genotype	26	300.36	11.55	58.83	0.00
Error	26	5.11	0.19		

Appendix I. (Cont'd)

I(d) :ANOVA for the response variable Yield per Plot

Item(s)	DF	Sum of Square	Mean Square	F value	P value
Replication	1	2.53	2.53	0.36	0.55
Genotype	26	10813.14	415.89	58.83	0.00
Error	26	183.81	7.06		

Appendix II. Pairwise mean differences among the genotype on the basis of LSD value 10.94

Genotype pair	**Mean difference**	**Probability**
Ashary-Bogra local	31.50	0.00
Ashary-Comilla local	11.10	0.00
Ashary-Cricket	13.50	0.01
Ashary-Dahazari	21.00	0.00
Ashary-Iswardi	30.00	0.00
Ashary-Jessore local-1	13.50	0.01
Ashary-Khulna local-1	12.00	0.00
Ashary-Narsingdi Local	19.50	0.00
Ashary-Rangpur local-1	25.50	0.00
Ashary-Rangpur local-2	13.50	0.01
Ashary-Sada Khulna	13.50	0.01
Ashary-Thakurgaon local	22.50	0.00
Ashary-Wild species (*S. torvum*)	47.70	0.00
Ashary-Wild species (*S. sisymbriifolium*)	47.25	0.00
Comilla Local-Bogra local	20.40	0.00
Cricket-Bogra Local	18.00	0.00
Dinajpur Local-Bogra Local	27.00	0.00
Jamalpur Local-Bogra Local	22.50	0.00

Appendix II. (Cont'd). Pairwise mean differences among the genotype on the basis of LSD value 10.94

Genotype pair	Mean difference	Probability
Jessore Local-1-Bogra Local	18.00	0.00
Jessore Local-2-Bogra Local	27.00	0.00
Jessore Local-3-Bogra Local	21.00	0.00
Jessore Local-4-Bogra Local	25.50	0.00
Khotkhotia-Bogra Local	27.00	0.00
Khulna Local-1-Bogra Local	19.50	0.00
Kurrigram Local-Bogra Local	34.50	0.00
Lalmoni Local-1-Bogra Local	37.50	0.00
Lalmoni Local-2-Bogra Local	31.50	0.00
Nilphamari local-Bogra Local	37.50	0.00
Narsingdi local-Bogra Local	12.00	0.02
Rangpur Local-2-Bogra Local	18.00	0.00
Rangpur Local-3-Bogra Local	22.50	0.00
Sada Khulna-Bogra Local	18.00	0.00
Salta Begun-Bogra Local	39.00	0.00
Bogra Local-Wild species (*S. torvum*)	16.20	0.00
Bogra Local –Wild Species (*Solanum sisymbrifolium*)	15.75	0.00
Comilla Local-Iswardi	18.90	0.00
Kurrigram Local-Comilla Local	14.10	0.00
Lalmoni Local-1-ComillaLocal	17.10	0.00
Lalmoni Local-2-Comilla Local	11.10	0.04
Nilphamari local-Comilla Local	17.10	0.00
Comilla Local-Rangpur Local-1	14.40	0.00
Salta Begun-Comilla Local	18.60	0.00
Comilla Local-Thakurgaon local	11.40	0.03
Comilla Local-Wild species (*S. torvum*)	36.60	0.00
Comilla Local-Wild species (*S. sisymbriifolium*)	36.15	0.00
Cricket-Iswardi	16.50	0.00
Kurrigram Local-Cricket	16.50	0.00

Appendix II. (Cont'd). Pairwise mean differences among the genotype on the basis of LSD value 10.94

Genotype pair	Mean difference	Probability
Lalmoni Local-1-Cricket	19.50	0.00
Lalmoni Local-2-Cricket	13.50	0.01
Nilphamari local-Cricket	19.50	0.00
Cricket-Rangpur Local-1	12.00	0.02
Salta Begun-Cricket	21.00	0.00
Cricket-Wild species (*S. torvum*)	34.20	0.00
Cricket-Wild Species(*Solanum sisymbrifolium*)	33.75	0.00
Dinajpur Local-Dohazari	16.50	0.00
Dinajpur Local-Iswardi	25.50	0.00
Dinajpur Local-Narsingdi local	15.00	0.00
Dinajpur Local-Rangpur Local-1	21.00	0.00
Salta Begun-Dinajpur Local	12.00	0.02
Dinajpur Local-Thakurgaon local	18.00	0.00
Dinajpur Local-Wild species (*S. torvum*)	43.20	0.00
Dinajpur Local-Wild Species(*Solanum sisymbrifolium*)	42.75	0.00
Jamalpur Local-Dohazari	12.00	0.02
Jessore Local-2-Dohazari	16.50	0.00
Jessore Local-4-Dohazari	15.00	0.00
Khotkhotia-Dohazari	16.50	0.00
Kurrigram Local-Dohazari	24.00	0.00
Lalmoni Local-1-Dohazari	27.00	0.00
Lalmoni Local-2-Dohazari	21.00	0.00
Nilphamari local-Dohazari	27.00	0.00
Rangpur Local-3-Dohazari	12.00	0.02
Salta Begun-Dohazari	28.50	0.00
Dohazari-Wild species (*S. torvum*)	26.70	0.00
Dohazari-Wild Species(*Solanum sisymbrifolium*)	26.25	0.00
Jamalpur Local-Iswardi	21.00	0.00

Appendix II. (Cont'd). Pairwise mean differences among the genotype on the basis of LSD value 10.94

Genotype pair	Mean difference	Probability
Jessore Local-1-Iswardi	16.50	0.00
Jessore Local 2- Iswardi	25.50	0.00
Jessore Local-3-Iswardi	19.50	0.00
Jessore Local-4-Iswardi	24.00	0.00
Khotkhotia-Iswardi	25.50	0.00
Khulna Local-1-Iswardi	18.00	0.00
Kurrigram Local-Iswardi	33.00	0.00
Lalmoni Local-1-Iswardi	36.00	0.00
Lalmoni Local-2-Iswardi	30.00	0.00
Nilphamari local-Iswardi	36.00	0.00
Rangpur Local-2-Iswardi	16.50	0.00
Rangpur Local-3-Iswardi	21.00	0.00
Sada Khulna-Iswardi	16.50	0.00
Salta Begun-Iswardi	37.50	0.00
Iswardi-Wild species (*S. torvum*)	17.70	0.00
Iswardi-Wild species (*S. sisymbriifolium*)	17.25	0.00
Kurrigram Local-Jamalpur Local	12.00	0.02
Lalmoni Local-1-Jamalpur Local	15.00	0.00
Nilphamari local-Jamalpur Local	15.00	0.00
Jamalpur Local-Rangpur Local-1	16.50	0.00
Salta Begun-Jamalpur Local	16.50	0.00
Jamalpur Local-Thakurgaon local	13.50	0.01
Jamalpur Local-Wild species (*S. torvum*)	38.70	0.00
Jamalpur Local-Wild Species(*Solanum sisymbrifolium*)	38.25	0.00
Kurrigram Local-Jessore Local-1	16.50	0.00
Lalmoni Local-1-Jessore Local-1	19.50	0.00
Lalmoni Local-2-Jessore Local-1	13.50	0.01
Nilphamari local-Jessore Local-1	19.50	0.00
Jessore Local-1-Rangpur Local-1	12.00	0.02

Appendix II. (Cont'd). Pairwise mean differences among the genotype on the basis of LSD value 10.94

Genotype pair	Mean difference	Probability
Salta Begun-Jessore Local-1	21.00	0.00
Jessore Local-1-Wild species (*S. torvum*)	34.20	0.00
Jessore Local-1-Wild Species(*Solanum sisymbrifolium*)	33.75	0.00
Jessore Local-2-Narsingdi local	15.00	0.00
Jessore Local-2-Rangpur Local-1	21.00	0.00
Salta Begun-Jessore Local-2	12.00	0.02
Jessore Local-2-Thakurgaon local	18.00	0.00
Jessore Local-2-Wild species (*S. torvum*)	43.20	0.00
Jessore Local-2-Wild Species(*Solanum sisymbrifolium*)	42.75	0.00
Kurrigram Local-Jessore Local-3	13.50	0.01
Lalmoni Local-1-Jessore Local-3	16.50	0.00
Nilphamari local-Jessore Local-3	16.50	0.00
Jessore Local-3-Rangpur Local-1	15.00	0.00
Salta Begun-Jessore Local-3	18.00	0.00
Jessore Local-3-Thakurgaon local	12.05	0.02
Jessore Local-3-Wild species (*S. torvum*)	37.20	0.00
Jessore Local-3-Wild Species(*Solanum sisymbrifolium*)	36.75	0.00
Lalmoni Local-1-Jessore Local-4	12.00	0.02
Nilphamari local-Jessore Local-4	12.00	0.02
Jessore Local-4-Narsingdi local	13.50	0.01
Jessore Local-4-Rangpur Local-1	19.50	0.00
Salta Begun-Jessore Local-4	13.50	0.01
Jessore Local-4-Thakurgaon local	16.50	0.00
Jessore Local-4-Wild species (*S. torvum*)	41.70	0.00

Appendix II. (Cont'd). Pairwise mean differences among the genotype on the basis of LSD value 10.94

Genotype pair	Mean difference	Probability
Jessore Local-4-Wild Species(*Solanum sisymbrifolium*)	41.25	0.00
Khotkhotia-Narsingdi local	15.00	0.00
Khotkhotia-Rangpur Local-1	21.00	0.00
Salta Begun-Khotkhotia	12.00	0.02
Khotkhotia-Thakurgaon local	18.00	0.00
Khotkhotia-Wild species (*S. torvum*)	43.20	0.00
Khotkhotia-Wild Species(*Solanum sisymbrifolium*)	42.75	0.00
Kurrigram Local-Khulna Local-1	15.01	0.00
Lalmoni Local-1-Khulna Local-1	18.00	0.00
Lalmoni Local-2-Khulna Local-1	12.00	0.02
Nilphamari local-Khulna Local-1	18.00	0.00
Khulna Local-1-Rangpur Local-1	13.50	0.01
Salta Begun-Khulna Local-1	19.50	0.00
Khulna Local-1-Wild species (*S. torvum*)	35.70	0.00
Khulna Local-1-Wild Species(*Solanum sisymbriifolium*)	35.25	0.00
Kurrigram Local-Narsingdi local	22.50	0.00
Kurrigram Local-Rangpur Local-1	28.50	0.00
Kurrigram Local-Rangpur Local-2	16.50	0.00
Kurrigram Local-Rangpur Local-3	12.00	0.02
Kurrigram Local-Sada Khulna	16.50	0.00
Kurrigram Local-Thakurgaon local	25.50	0.00
Kurrigram Local-Wild species (*S. torvum*)	50.70	0.00
Kurrigram Local-Wild species (*S. sisymbrifolium*)	50.25	0.00
Lalmoni Local-1-Narsingdi local	25.50	0.00
Lalmoni Local-1-Rangpur Local-1	31.50	0.00
Lalmoni Local-1-Rangpur Local-2	19.50	0.00

Appendix II. (Cont'd). Pairwise mean differences among the genotype on the basis of LSD value 10.94

Genotype pair	Mean difference	Probability
Lalmoni Local-1-Rangpur Local-3	15.00	0.00
Lalmoni Local-1-Sada Khulna	19.50	0.00
Lalmoni Local-1-Thakurgaon local	28.50	0.00
Lalmoni Local-1-Wild species (*S. torvum*)	53.70	0.00
Lalmoni Local-1-Wild Species(*Solanum sisymbrifolium*)	53.25	0.00
Lalmoni Local-2-Narsingdi local	19.50	0.00
Lalmoni Local-2-Rangpur Local-1	25.50	0.00
Lalmoni Local-2-Rangpur Local-2	13.50	0.00
Lalmoni Local-2-Sada Khulna	13.50	0.01
Lalmoni Local-2-Thakurgaon local	22.50	0.00
Lalmoni Local-2-Wild species (*S. torvum*)	47.70	0.00
LalmoniLocal-2-Wild species (*S. sisymbriifolium*)	47.25	0.00
Nilphamari local-Narsingdi local	25.50	0.00
Nilphamari local-Rangpur Local-1	31.50	0.00
Nilphamari local-Rangpur Local-2	19.50	0.00
Nilphamari local-Rangpur Local-3	15.00	0.00
Nilphamari local-Sada Khulna	19.50	0.00
Nilphamari local-Thakurgaon local	28.50	0.00
Nilphamari local-Wild species (*S. torvum*)	53.70	0.00
Nilphamari local-Wild species (*S. sisymbriifolium*)	53.25	0.00
Salta Begun-Narsingdi local	27.00	0.00
Narsingdi local-Wild species (*S. torvum*)	28.20	0.00
Narsingdi local-Wild species (*S. sisymbriifolium*)	27.75	0.00

Appendix II. (Cont'd). Pairwise mean differences among the genotype on the basis of LSD value 10.94

Genotype pair	Mean difference	Probability
Rangpur Local-2-Rangpur Local-1	12.00	0.02
Rangpur Local-3-Rangpur Local-1	16.50	0.00
Sada Khulna-Rangpur Local-1	12.00	0.02
Salta begun-Rangpur Local-1	33.00	0.00
Rangpur Local-1-Wild species (*S. torvum*)	22.20	0.00
RangpurLocal-1-Wild species (*S. sisymbriifolium*)	21.75	0.00
Salta Begun-Rangpur Local-2	21.00	0.00
Rangpur Local-2-Wild species (*S. torvum*)	34.20	0.00
Rangpur Local-2-Wild Species(*Solanum sisymbrifolium*)	33.75	0.00
Salta Begun-Rangpur Local-3	16.50	0.00
Rangpur Local-3-Thakurgaon local	13.50	0.01
Rangpur Local-3-Wild species (*S. torvum*)	38.70	0.00
Rangpur Local-3-Wild species (*S. sisymbriifolium*)	38.25	0.00
Salta Begun-Sada Khulna	21.00	0.00
Sada Khulna-Wild species (*S. torvum*)	34.20	0.00
Sada Khulna-Wild species (*S. sisymbriifolium*)	33.75	0.00
Salta Begun-Thakurgaon local	30.00	0.00
Salta Begun-Wild species (*S. torvum*)	55.20	0.00
Salta Begun-Wild species (*S. sisymbriifolium*)	54.75	0.00
Thakurgaon local-Wild species (*S. torvum*)	25.20	0.00
Thakurgaon local-Wild species (*S. sisymbriifolium*)	24.75	0.00