

# Molecular characterization and DNA fingerprinting of some local eggplant genotypes and its wild relatives

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**Abstract**— Collection and characterization of local genotypes and landraces are prerequisite for any crop improvement program. Molecular diversity and DNA profiling shown exact genetic blue print of any crop. Hence, the experiment was design to establish the molecular diversity and polymorphism among some local eggplant genotypes and its wild relatives for future breeding program. The experiment was carried out at the Biotechnology Laboratory, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, with twenty-five local and two wild relatives (*Solanum sisymbriifolium* and *S. villosum*) of eggplant to study molecular diversity and DNA fingerprinting at those genotypes. Five well-known SSR primers (EPSSR82, smSSR01, EM114, EM120 and smSSR04) were used for the molecular characterization of the genotypes. Quality DNA was isolated with 27 genotypes and PCR amplification was carried out with these primer. The amplified DNA fragment was visualized by 2% agarose gel and data were analyzed by POWERMAKER (version 3.25) and NTSYS-PC (version 2.2). Some total at 10 different alleles were generated with a range of 1 to 3 alleles per locus and an average of 2.0 alleles. The highest number (2) of polymorphic bands was observed in the primers EPSSR82 and smSSR01. The Polymorphism Information Content (PIC) of SSR markers ranged from 0.37 to 0.67 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. UPGMA method separated the of 27 genotypes into two major clusters (I and II). From the clusters, wild species *Solanum villosum* 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. Molecular diversity and DNA profiling was identified among 25 local eggplant germplasm and its wild relatives. The finding of the

experiment could be used for selection of diverse parent for eggplant improvement.

**Keywords**— eggplant, molecular diversity, SSR marker, wild relatives.

## I. INTRODUCTION

Eggplant (*Solanum melongena* L.  $2n = 2x = 24$ ) belongs to the plant family of Solanaceae. It is the sixth most important vegetable after tomato, watermelon, onion, cabbage, and cucumber and the most important *Solanum* crop native to Asia [1]. Eggplants have a remarkable demand and are considered as the second important vegetable crop after potato in Bangladesh [2]. 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 annual production of 4,50,146 metric tons [2]. Eggplant has a number of health benefits. It is an important source of fiber, potassium, manganese, as well as vitamins C, K, and B6. 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 anti-carcinogenic agent. Despite eggplant's economic importance, its improvement and molecular study of different land races, local genotypes and germplasm characterization was not well studied. The development of new eggplant varieties addressing old and new breeding objectives requires of genetic diversity [3, 4, 5]. Collection and characterization of genetic resources and local cultivars are required for the improvement of new varieties. 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 [6,7]. Co-dominant markers such as simple sequence repeat (SSRs) could generate more information and has high repeatability than other dominant markers like RAPD or AFLP [8,9]. SSRs have proved as a more powerful marker than AFLPs to study the relationships amongst closely related eggplant materials [10]. SSR markers are multi-allelic, highly abundant, well distributed in the genome and are suitable for high throughput PCR which makes them ideal for molecular diversity studies [11]. 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. Wild species remain largely unexploited for eggplant breeding. *S. villosum* and *S. sisymbriifolium* are two wild relatives of *Solanum melongena* which showed considerable resistance to bacterial wilt. So, the study was focused on genetic diversity of some local eggplant germplasm through SSR marker to generate more information and to assess relatedness among local landraces and also with their wild relatives.

## II. MATERIALS AND METHOD

### Collection of material

A sum total of 27 materials were used in the study and among those 25 were local eggplant genotypes and 2 were wild relatives viz. *Solanum villosum* and *S. sisymbriifolium*). Germplasms were collected from different districts of Bangladesh. A list of local germplasm and their collected area was given in Table 1. Wild relatives were collected from the Gene Bank of Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. The experiments were carried out at the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, Bangladesh.

### Seedling raising

Good quality, disease free, healthy seed were sown in plastic pots and kept in nets house. All management practices were done for raising quality seedlings from those materials. Fresh leaves were collected at 3-4 leaf stage of plant for isolation of DNA.

### Extraction and quantification of DNA

Total genomic DNA from each genotypes was isolated by CTAB method with slight modification [12]. The extracted DNA was purified by propanol and treated with 10µg/ml RNase A for 20-25 minutes at 37°C to remove the RNA. The purified DNA was dissolved in TE buffer and quantification of DNA was done through electrophoresis on 1% agarose gel staining by ethidium bromide. The sample DNA were stored at -20°C freezer for further use.

### Primer selection and PCR amplification

Five SSR primers were selected on the basis of previous works to evaluate the molecular polymorphism study the eggplant local genotypes and wild relatives. PCR reaction was performed using BIONEER KIT (Korea). The PCR reaction having 20.0 µl mixture containing with 3.0 µl sterile de-ionized water, 4.0 µl 10X PCR buffer, 4.0 µl enzyme dilution buffer, 3.0 µl 20 mM MgCl<sub>2</sub>, 1.0 µl dNTPs (10mM), 0.5 µl top DNA polymerase, 2.5 µl primer (forward and reversed) and 2.0 µl sample DNA (approx. 40-50 ng). The reaction mixture was subjected to the following thermal profile for amplification in a thermocycler: 5.00 min at 95°C for initial denaturation, followed by 33 cycle of 1.00 minutes denaturation at 94°C, 1.00 minutes at annealing with various temperature according to primer melting point and 1.30 minutes at 72°C for extension. A final extension step was done at 72°C for 7 minutes. Electrophoresis was done to visualize the PCR amplified product. It was carried out on 2.0% agarose gel and amplified fragments were visualized by staining with ethidium bromide.

### Documentation of PCR amplified DNA products and SSR data analysis

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. 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) [13]. 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 [14,15]. Unweighted Pair Group Method of Arithmetic Means (UPGMA) dendrogram was constructed using a computer programme, POPGENE (Version 1.31) based on Nei's [16] genetic distance.

## III. RESULTS

Eggplant (*Solanum melongena*) is an important vegetable in our country which has 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. 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.

#### 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 2 and Fig. 1 to 4.

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 Fig. 1.

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 band (Fig. 2). The SSR primer smSSR01 was able to amplify three fragments of DNA among all the individuals. The DNA product ranged from 200 to 350 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 villosum* produced third amplification at 250 bp, which was polymorphic (Fig. 3). 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 (Fig. 04). On an average, five SSR primers were able to generate some total of 10 DNA amplification (10 bands) with an average amplification for each primer was 2.0. Out of them, five DNA fragment were polymorphic among the genotypes under studied.

#### 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 3. PCR products of five SSR markers were characterized. Some total 10 alleles were detected for the five polymorphic SSR loci, with an average number of alleles/locus is 2.0. The frequency of the major allele ranged between 0.33 to 0.56 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. Highest PIC value (0.67) was observed in the primer EPSSR82 and it was lowest (0.37) in the primer smSSR01. The primer EPSSR82 was considered as the best marker for diversity analysis in eggplant germplasm followed by EM114 and EM120, respectively. The marker smSSR04 was considered as the least powerful marker. Gene diversity ranged between 0.49 (smSSR 01) to 0.72 (EPSSR 82) with an average of 0.61. The results indicate that the 25 local eggplant landraces present a high degree of homozygosity and are closely related to the wild variety *Solanum villosum* and *Solanum sisymbriifolium*, and also considerable intra-varietal group diversity, and a certain degree of genetic differentiation and polymorphism really do exist.

#### Nei's Genetic Distance and Genetic Identity

The value of pair-wise comparisons of Nei's (1972) genetic distance (D) among twenty-five local and two wild relatives of eggplant were computed from combined data of the five primers and it was 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 and two wild species. The wild species *Solanum villosum* showed highest genetic distance among twelve eggplant genotypes. The highest genetic distance between them indicated that genetically they are diversified. Genotypes pair with higher value (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 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. 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.

#### 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 (Fig. 5). Ashary, Khotkhotia, Kurigram local, Lalmoni local-1, Lalmoni local-2, Salta begun, Cricket, Dinajpur local, Nilphamari local, Rangpur-1, Rangpur-2, Rangpur-3 were 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*) were fallen in the cluster II(a) and only one wild species (*Solanum villosum*) formed cluster-II (b). The genotypes – Ashary, Khotkhotia, Kurigram local, Lalmoni local-1, Lalmoni local-2, Salta begun were formed cluster I(a) and the germplasm Cricket, Dinajpur local, Nilphamari local, Rangpur-1, Rangpur-2, Rangpur-3 formed cluster-I(b). 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 was clearly observed that wild species (*Solanum villosum*) was very much different from all the genotypes. The result indicates that the low or high level genetic distance exists within the genotypes.

#### IV. DISCUSSION

Eggplant is an important vegetable crop in Bangladesh. Different local genotypes and wild relatives were found in Indian sub-continent. Morphologically those genotypes showed huge variation. Diversity study through molecular marker expressed the actual genetic make-up of eggplant genotypes. The present observation noticed the polymorphism at DNA level among the twenty-five local and two wild relatives. This finding also supported by various scientist. Some of them were discussed below.

The 22 amplified DNA products using nine SSR primers with an average amplification for each primer of 2.2 were noticed in six eggplant genotypes showed 70% monomorphic and 30% polymorphic band through using 9 primers in eggplant genotypes [17]. Nineteen SSRs markers for the molecular characterization of 30 eggplant accessions were studied. The polymorphism information content (PIC) of SSR markers ranged from 0.07 to 0.77, with an average value of PIC=0.50[18]. The mean observed heterozygosity ( $H_o$ ) presented a very low value  $H_o=0.01$ , while the mean expected heterozygosity ( $H_e$ ) had a value of  $H_e=0.57$ . Genomic SSRs that previously proved to be highly polymorphic in eggplant have been

found to be of great value for evaluating the genetic diversity and relationships in a collection of eggplants from different cultivar groups [18, 19]. It possesses a number of desirable horticultural traits such as disease resistance [20] and has medicinal uses [21]. This result clearly indicated that different levels of genetic identity and distance present within the eggplant germplasm and shown in the UPMGA dendrogram (Fig. 5). Different levels of cluster analysis was reviewed which was performed by several scientists. 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[22]. An experiment with 19 SSR markers to analysis genetic diversity among 30 Spanish eggplant genotypes revealed a considerable diversity exists within each of the cultivar groups. Germplasm from different regions shows a wide range of genetic diversity as well as phenotypic diversity indeed.

#### V. 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. 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. 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. Dendrogram figure revealed that, the 25 local and two wild relatives of eggplant into two major clusters. 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 improvement of cultivars. Molecular characterization of local eggplant data might be helpful to select the diverse parents for development of a new variety.

#### SIGNIFICANT STATEMENT

This work able to identify polymorphism among local genotypes through SSR markers. Molecular diversity and genetic distance also established between cultivated eggplant and its wild relatives viz. *Solanum villosum* and *Solanum sisymbriifolium*. The result may utilized as a source of diverse parent for any hybridization program.



Diversity at DNA level information will be used to conserve the local germplasm for future use.

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#### REFERENCES

- [1] FAO (2016). FAOSTAT Production Databases. Available online at: <http://faostat3.fao.org/home/E>(Accessed 11 February, 2016).
- [2] BBS. 2016. Statistical Yearbook-2015. Bangladesh Bureau of Statistics, Ministry of Planning, Government of People's Republic of Bangladesh.
- [3] Barchi, L., S. Lanteri, E. Portis, G. Valè, A. Volante, L. Pulcini, and G. L. Rotino, 2012. A RAD tag derived marker based eggplant linkage map and the location of QTLs determining anthocyanin pigmentation. *PLoS One*. 7: e43740.
- [4] Lebeau, A., M. Gouy, M. C. Daunay, E. Wicker, F. Chiroleu, P. Prior and J. Dintinger, 2013. Genetic mapping of a major dominant gene for resistance to *Ralstonia solanacearum* in eggplant. *Theor. Appl. Genet.* 126: 143-158.
- [5] Sunseri, F., A. Sciancalepore, G. Martelli, N. Acciari, G.L. Rotino, D. Valentino, and G. Tamietti, 2003. Development of RAPD-AFLP map of eggplant and improvement of tolerance to *Verticillium* wilt. In: Hammerschlag FA, Saxena P [eds] XXVI International Horticultural Congress: Biotechnology in Horticultural Crop Improvement. *Acta Hortic.* 625: 107-115.
- [6] Nunome, T., K. Suwabe, H. Ketani, and M. Hirai, 2003. Identification and characterization of microsatellites in eggplant. *Plant Breed.* 122: 256-262.
- [7] Stàge, A., E. Portis, L. Toppino, G. L. Rotino, and S. Lanteri, 2008. Gene-based microsatellite development for mapping and phylogeny studies in eggplant. *BMC Genome.* 9 : 357.
- [8] Nunome, T., S. Negoro, I. Kono, H. Kanamori, and K. Miyatake, 2009. Development of SSR markers derived from SSR-enriched genomic library of eggplant (*Solanum melongena* L.). *Theor. Appl. Genet.* 119: 1143-1153.
- [9] Fukuoka, H., H. Yamaguchi, T. Nunome, S. Negoro, K. Miyatake, and A. Ohyama, 2010. Accumulation, functional annotation, and comparative analysis of expressed sequence tags in eggplant (*Solanum melongena* L.), the third pole of the genus *Solanum* species after tomato and potato. *Gene.* 450: 76-84.
- [10] Muñoz-Falcón, J. E., J. Prohens, S. Vilanova, F. Ribas, A. Castro, and F. Nuez, 2009. Distinguishing a protected geographical indication vegetable (Almagro eggplant) from closely related varieties with selected morphological traits and molecular markers. *J. Sci. Food Agric.* 89: 320-328.
- [11] Powell, W., G. C. Machray, and J. Provan, 1996. Polymorphism revealed by simple sequence repeats. *Trends Plant Sci.* 1: 215-222.
- [12] Frey, J.E., B. Frey, C. Sauer, and M. Kellerhals, 2004. Efficient low cost DNA extraction and multiplex fluorescent PCR method for marker assisted selection in breeding. *Plant Breed.* 123:554-557.
- [13] Liu, K. and S. V. Muse, 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics.* 21: 2128-2129.
- [14] Anderson, J.A., G.A. Churchill, J.E. Autrique, S.D. Tanksley and M.E. Sorrells, 1993. Optimizing parental selection genetic linkage maps. *Genome.* 36:181-186.
- [15] Rohlf, F. J. 2001. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System, Version 2.02. Setauket, New York.
- [16] Nei, M. 1972. Genetic distance between populations. *Am. Nat.* 106: 283-292.
- [17] Khorsheeduzzaman, A. K. M., M. Z. Alam, M. M. Rahman, M. A. K. Mian, M. I. H. Mian, and M. M. Hossain, 2008. Molecular characterization of five selected brinjal (*Solanum melongena* L.) genotypes using SSR markers. *Bangladesh J. Plant Breed. Genet.* 21: 01-06.
- [18] Vilanova, S., M. Hurtado, A. Cardona, M. Plazas, F. J. Herraiz, P. Gramazio and J. Prohens, 2014. Genetic diversity and relationships in local varieties of eggplant from different cultivar groups as assessed by genomic SSR markers. *Not. Bot. Horti. Agrobot. Cluj-Napoca.* 42: 59.
- [19] Vilanova, S., J.P. Manzur, and J. Prohens, 2012. Development and characterization of genomic simple sequence repeat markers in eggplant and their application to the study of diversity and relationships in a collection of different cultivar types and origins. *Mol. Breed.* 30: 647-660.
- [20] Kumchai, J., Y. C. Wei, C. Y. Lee, F. C. Chen, and S. W. Chin, 2013. Production of interspecific hybrids between commercial cultivars of the eggplant (*Solanum melongena* L.) and its wild relative *S. torvum*. *Genet. Mol. Res.* 12: 755-64.
- [21] Yousaf, Z., Y. Wang and E. Baydoun, 2013. Phytochemistry and pharmacological studies on

Solanum torvum Swartz. J. Appl. Pharm. Sci. 3: 152-16.  
 [22] Caguiat, X. G. I. and D. M. Hautea, 2014. Genetic diversity analysis of eggplant (*Solanum melongena*

L.) and related wild species in the Philippines using morphological and SSR markers. SABRAO J. Breed. Genet. 46: 183-201

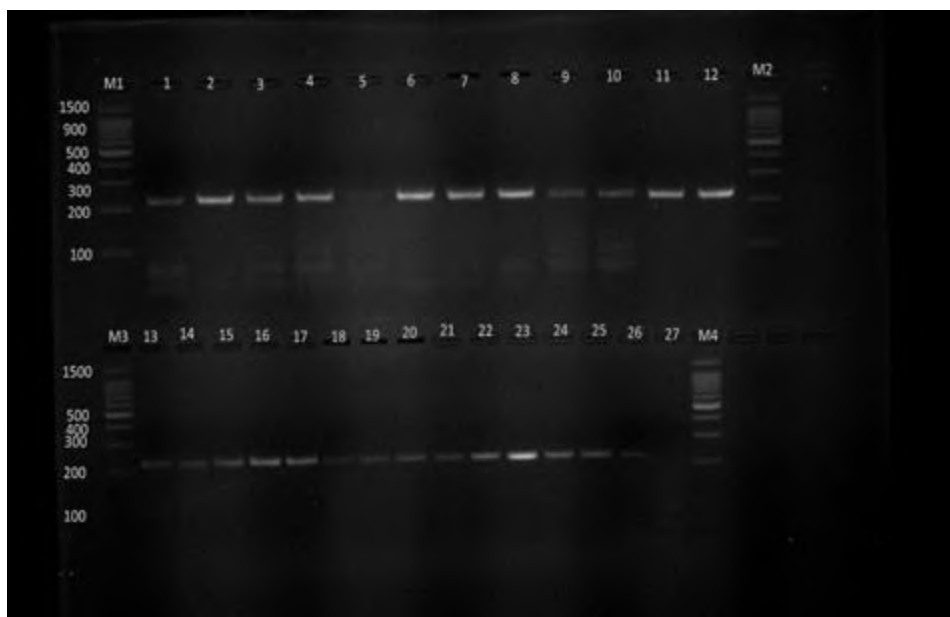


Fig. 1: SSR profile of 27 local and wild eggplant germplasm using primer EM114.

(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; Lane 11: 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 villosum* and M1=M2=M3=M4=100 bp DNA ladder).

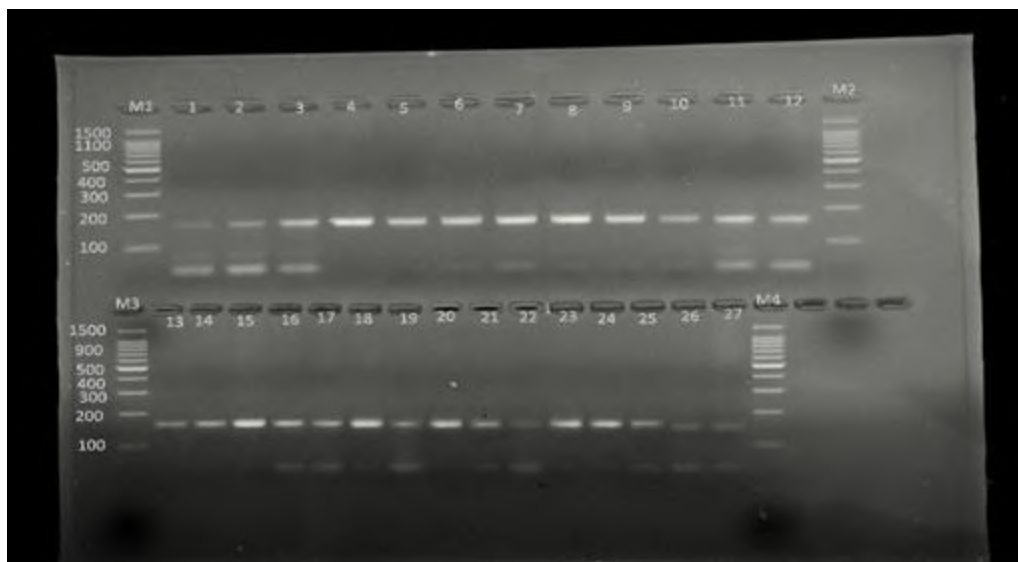


Fig. 2: SSR profile of 27 local and wild eggplant germplasm using primer EM120.

(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; Lane 11: 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 villosum* and M1=M2=M3=M4=100 bp DNA ladder).

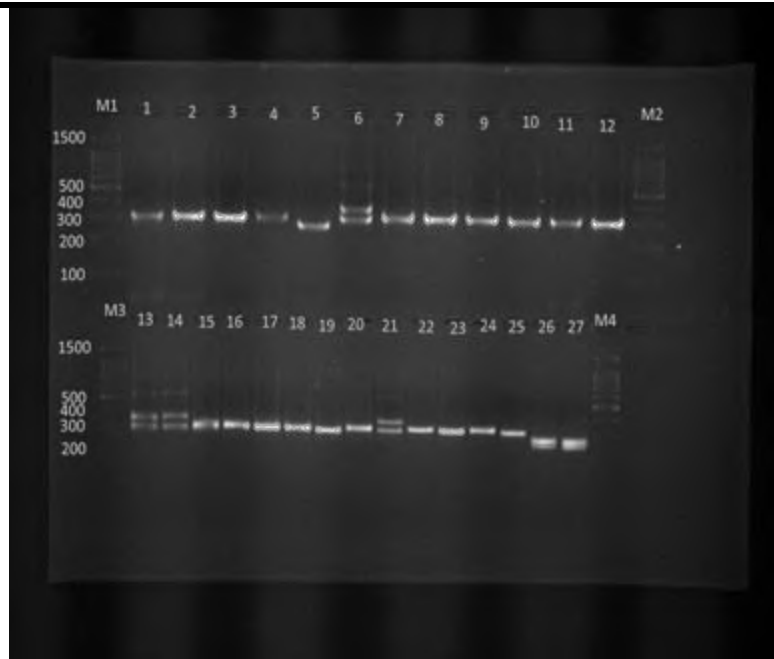


Fig. 3: SSR profile of 27 local and wild eggplant germplasm using primer smSSR01.

(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; Lane 11: Nilphamari local; Lane 12: Dinajpur local ; Lane 13: Thakurgaon local; Lane 14: Bogra local; Lane 15: Iwardi 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 villosum* and  $M1=M2=M3=M4=100$  bp DNA ladder.

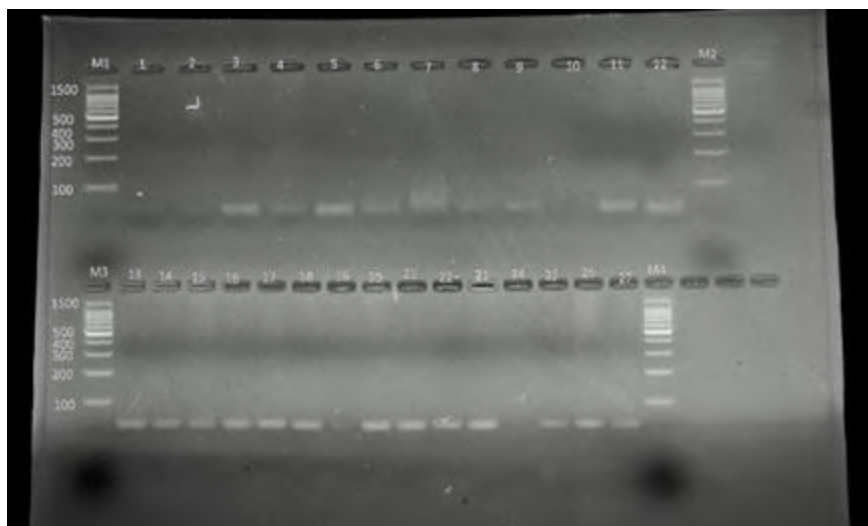


Fig. 4: SSR profile of 27 local and wild eggplant germplasm using primer smSSR04.

(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; Lane 11: Nilphamari local; Lane 12: Dinajpur local ; Lane 13: Thakurgaon local; Lane 14: Bogra local; Lane 15: Iwardi 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 villosum* and  $M1=M2=M3=M4=100$  bp DNA ladder.

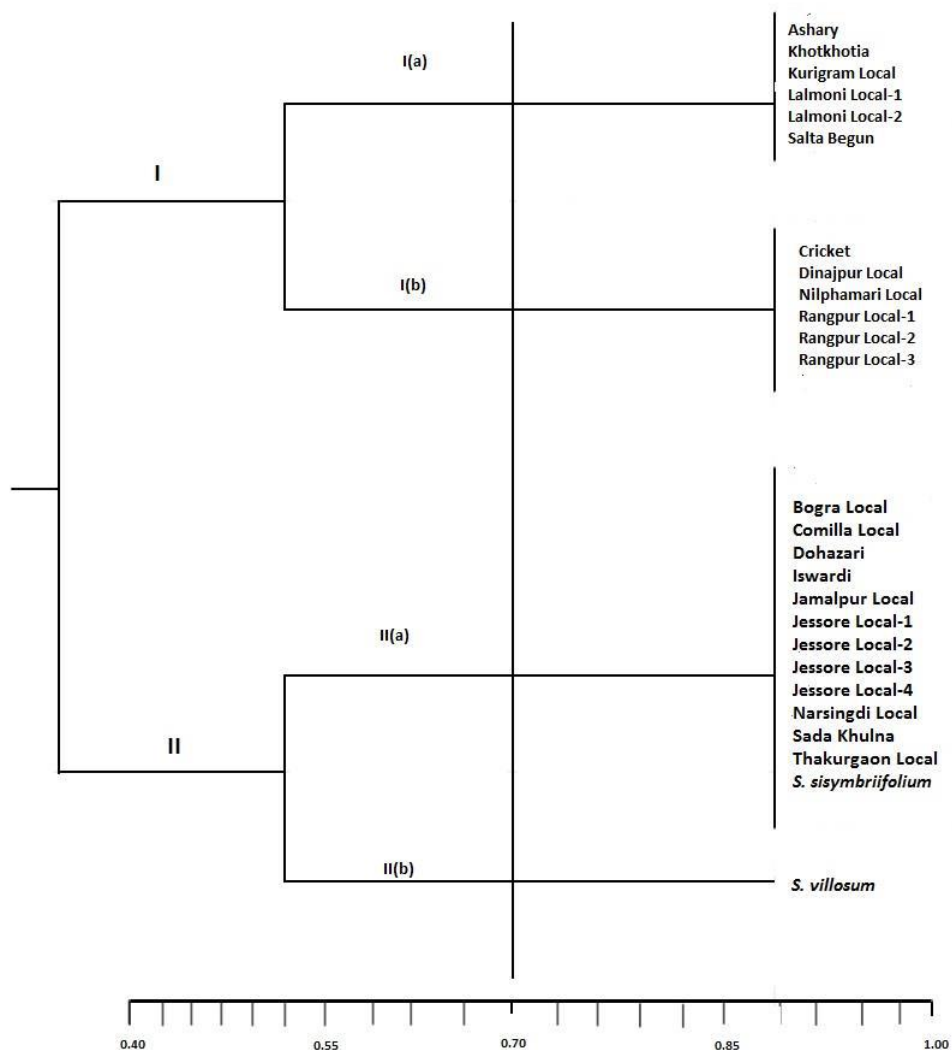


Fig.5: Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's (1972) genetic distance, summarizing data on differentiation for twenty-five local and two wild relatives of eggplant.

Table 1: Name of the local genotypes and their collected area in Bangladesh.

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 ( <i>Solanum</i> <i>sisymbriifolium</i> )		*BARI, Gazipur	
27	Wild species ( <i>Solanum villosum</i> )		*BARI, Gazipur	

\*BARI= Bangladesh Agricultural Research Institute

Table.2: PCR amplified DNA 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 range (bp)
1	EM114	For. AGCCTAAACTTGGTTGGTTTTTGC Rev. GAAGCTTTAAGAGCCTTCTATGCA G	43	1	0	225
2	EM120	For. GGATCAACTGAAGAGCTGGTGGTT Rev. CAGAGCTTCAATGTTCCATTTAC A	44	2	1	50-180
3	EPSSR82	For. ACATGCCACTCATGTTGGTG Rev. CTCAGCCATGGACCACATT	50	3	2	50-180
4	smSSR01	For. GTGACTACGGTTTCACTGGT Rev. GATGACGACGACGATAATAGA	46	3	2	200 - 400
5	smSSR04	For. AATGAGTCAGAAACCACGCC Rev. CGTTTAAACCTTTGCTCGGAA	49	1	0	50-80
Total	-	-	-	10	5	-
Mean	-	-	-	2.0	1.0	-

Table.3: Major allelic frequency, gene diversity and PIC value of different eggplant 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