INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 19, No. 3, p. 157-165, 2021

RESEARCH PAPER OPEN ACCESS

Anatomical and genetical variability of brinjal (*Solanum melongena* L.) varieties based on RAPD marker

S.N Sima*, Irin Lithy

Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh

Key words: *Solanum melongena* L., Anatomical character, Genetic diversity, RAPD, Cluster Analysis.

http://dx.doi.org/10.12692/ijb/19.3.157-165 Article published on September 30, 2021

Abstract

The present investigation was aimed at studying the anatomical characters, quantitative measurement and anatomical features of the stem and leaf of five local varieties Golmakra, Debjhuri hajari, Golapi F1, Kanta begun and China of *Solanum melongena* of Solanaceae family. Genetic variation was assessed by RAPD DNA fingerprinting method with different primers. The anatomical investigation was conducted by cutting transverse slices from the materials, staining them using double-staining methods, and observing the findings using a powerful compound microscope. Four primers and RAPD molecular markers were used for the molecular analysis. The epidermis was documented as a single layer, according to anatomical study. In the stem, bi-collateral vascular bundles were visible and typically aligned in radial symmetry. RAPD based dendrogram were generated by the UPGMA method and cluster analysis revealed that distinct clusters were found. The preliminary results indicated both genetical and anatomical relationship of stem and leaf of brinjal varieties could be potential sources of germplasm for their use in variety improvement.

* **Corresponding Author:** S.N Sima ✉ sima_bot2006@yahoo.com

Introduction

Brinjal *(Solanum melongena L.)* is widely cultivated as an important edible vegetable crop in both temperate and tropical areas. Brinjal (*S. melongena L.*) is an economically important Solanaceous vegetable which is widely consumed in Asia, Europe, Africa, and America (Ali *et al.,* 2011, Kumar *et al.,* 2008; Collonnier *et al.,* 2003). Its early European name is 'eggplant' and locally known as 'Begon' and also known as guinea squash, garden egg and aubergine (Khan *et al.,* 2013). It belongs to the Solanaceae family and is normally a self-pollinated, diploid (2n=2x=24) annual crop (Thompson, 1951). Solanaceae is a large plant family with more than 3,000 species and genus Solanum, a close relative of potato, tomato and pepper (Spooner and Knapp, 2013; Barrell *et al.,* 2013). In Bangladesh, brinjal is one of the most important vegetable crops which grown round the year in every corner of the country. Next to the potato brinjal is the second most important vegetable crop of Bangladesh in respect of acreage and production (BBS, 2012). It is also one of the widely used vegetable crops and is popular in many countries viz. Central, South and South East Asia, some parts of Africa and Central America (Harish *et al.,* 2011). As an ancient crops the cultivated brinjal is undoubtedly of Indian origin and has been in cultivation for a long time (Thompson and Kelly, 1957). Hundreds of cultivars with many wild types are available in this area (Sing *et al.,* 2001). The secondary centers of brinjal origin are Indo-Burma, China and Japan (Gleddie *et al.,* 1986). Duringm the 15th century Arabs introduced brinjal to the west (Hinata, 1986). The medicinal and economic value of brinjal is found in Sanskrit literature (Kalloo, 1993; Hinata, 1986; Khan, 1979). Brinjal germplasm resources and collections have been well documented, evaluated and conserved throughout the world (Sarathbabu *et al.,* 1999).

It is a nutritious vegetable and has got multifarious uses as a dish item (Rashid, 1993). It has higher calories, iron, phosphorus and riboflavin contents than tomato (Shaha, 1989). The fruit and other parts of the plant are used in traditional medicine.(Kashyap *et al.,* 2003). For example, tissue extracts have been used for the treatment of asthma, bronchitis, cholera and dysuria; fruits and leaves are beneficial in lowering blood cholesterol.It has potential health effects against cancer, aging, inflammation, and neurological diseases.Brinjal is susceptible to several pest sand diseases as well as abiotic stress conditions (Kashyap *et al.,* 2003; Sihachakr *et al.,* 1994).In contrast, the majority of wild species are resistant to nearly all known pests and pathogens of brinjal and thereby are a source of desirable traits for crop improvement. Plant breeders have addressed these constraints by identifying resistant or tolerant germplasm, determining the genetics involved and the genetic map positions of the resistant genes. Molecular markers are reliable tools to characterize the DNA profile of plant genotypes to study the genetic diversity. Molecular markers can provide information that can helps to define the distinctiveness of species and their ranking according to the number of close relatives and their phylogenetic position. For these reasons, molecular markers are rapidly being adopted by crop improvement research globally as an effective and appropriate tool for basic and applied studies addressing biological components in agricultural production systems (Jones *et al.,* 1997).

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

cultivars by PCR based RAPD technique, as it is important particularly for variety selection for breeding purpose such as, hybridization, evaluation and conservation of their diverse gene pool.

Materials and methods

Anatomical studies

Five eggplant cultivars Golmakra, Kanta begun, Golapi-F1, China, Debjhuri hajari were collected from local market of Rajshahi and seedling were grown in Department of Botany, University of Rajshahi. Stem and leaf were collected from the five varieties at their flowering time. The free hand transverse section was cut by a razer blade and thin sections were separated with the help of a research microscope. The selected thin sections were then stained with double stain technique (Johanson, 1940). Sectional anatomy was examined through a research microscope fitted with digital camera. Gross anatomy were studied by taking microphotographs and quantitative measurements of various tissues, cell etc. were described and analyzed to draw conclusion and probable interpretation of the system.

Genetical studies

Total genomic DNA from young leaves of young plants were isolated from the five brinjal varieties following chloroform: isoamyl alcohol purification and propanol precipitation method (Doyle and Doyle 1987). The isolated samples were stored at –20°C. DNA concentrations were determined at 260 nm with spectrophotomer (Nano Drop) Genetic Relationship Among five promising brinjal Varieties and the quality verified by electrophoresis on 1% agarose gel in TAE (Trisacetate-EDTA) buffer. PCR conditions were optimized by varying the concentrations of template DNA, Taq DNA polymerase and $MgCl_2$ concentration. Initial screening was done with 20-mer primers (Operon Technologies Inc., USA) using from each genomic DNA sample.

The primers that gave reproducible and scorable amplifications were used in the analysis of all the five varieties are OPW-04, OPW-09, OPW-10, and OPW-16. Total reaction volume for DNA amplification was 25 µl. Master mix for PCR reaction was prepared using sddH2O, Buffer A (10X) with 15 mM MgCl2, dNTPs (100mM of dATP, dCTP, dGTP and dTTP), template DNA and TaqDNA polymerase (5U/µl).DNA amplification was performed (Thermal Cycler, Eppendrop) as follows:1 cycle of 3 min at 95°C (initial strand separation) followed by 42 cycles each of 30 sec at 95°C (denatuartaion), 25 sec at 32°C (annealing) and 60 sec at 72°C (primer extension) after the last cycle, a final step of 5 minutes at 72°C was added to allow complete extension of all amplified fragments. After amplification, PCR products were stored at 4°C untill electrophoresis. Reaction products were mixed with 2.5 µl of 10X loading dye (Sambrook *et al.*1989) and spin briefly in a micro centrifuge before loading. PCR products were resolved by electrophoresis at 1% agarose gel, 120 V for 1.5 hr followed by staining with ethidium bromide. Gels were photographed in Gel Documentation System (Alphainnotech, USA).

Since RAPD markers are dominant, it is assumed that each band represented the phenotype at a single allelic locus (Williams *et al.* 1990).

All distinct bands or fragment (RAPD markers) were thereby given identification numbers according to their position on gel and scored visually on the basis of their presence (1) or absence (0), separately for each individual and each primer.

The scores obtained using all the primers in the RAPD analysis were then combined to create a single data matrix, to estimate linkage distance (D) and to construct a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among populations using a computer program, "Statistica".Linkage distances were computed from frequencies of polymorphic marker to estimate genetic relationship between the studied five brinjal varieties using the unweighted pair- group method of arithmetic means (UPGMA) (Sneath and sokal, 1973). The dendrogram was constructed using the “statistica” computer software.

Results and discussion

Anatomical studies

Transverse section of stem

The transverse section of stem showed single layered epidermis which was the outermost protective zone consisting of cells which were flattened tangentially butj had nearly similar radial length. The outer periclinal wall of the epidermal cells was much thicker due to the presence of cuticles. The epidermal out epidermal growths, multicellular hairs were present which was simple in types and uniseriate. Cortex consists of 6-7 layers of collenchyma cells and 5-7 layers of parenchyma cells. The parenchyma cells were isodiametric with thin wall and with intercellular spaces. The inner most single layer endodermis was present which acted as limiting layers of the cortex and the vascular cylinder. The pericycle consisted of 1-2 layers of sclerenchyma cells. The vascular bundles are situated inside the pericycle which consists of xylem and phloem.

They were bi-collateral type and arranged in ring like shape towards the periphery.Phloem consisted of sieve tubes and sieve plates. Companion cells were observed in contact with sieve tubes. A considerable amount of phloem parenchyma was observed. Xylem consisted of metaxylem, protoxylem and parenchyma. The pith was made up with parenchymatous cells having thin walls and prominent intercellular spaces.

Table 1. Mean with standard error of Epidermal Cell Length Radial (ECLR), Epidermal Cell Length Tangential (ECTL), Epidermis Area(EA), Vascular Bundle Area (VBA), Xylem Area (XA), and Phloem Area (PA).

Variety	Item	ECLR	ECLT	EA	VBA	XA	PA
Golmakra	Stem	1.14±0.05	2.10±0.07	565.58□10.29	2500.34□88.83	1262.71□48.57	960.84□45.98
	Midrib	1.14±0.11	2.06±0.11	493.90□7.24	1477.32□106.69	779.47□27.00	808.54□44.41
Debjhuri hajari	Stem	1.22±0.08	2.20±0.14	580.36□7.54	2656.36□73.07	1272.48□45.85	965.68□33.72
	Midrib	1.12±0.04	1.98±0.13	481.94□6.38	1600.78□106.17	786.50□26.58	782.02□53.25
Golapi-F1	Stem	1.18±0.08	1.96±0.11	548.72□9.16	2049.70□137.86	1194.02□38.62	816.24□24.79
	Midrib	1.12±0.08	2.14±0.09	515.54□7.87	1574.92□119.92	888.76□41.30	774.42□55.06
Kanta	Stem	1.14±0.05	2.02±0.13	582.82□7.46	1917.80□154.51	1216.75□37.82	857.82□47.08
	Midrib	1.20±0.07	2.10±0.19	438.36□10.95	1503.44□102.60	817.40□35.30	755.78□42.22
China	Stem	1.14±0.09	2.02±0.13	532.02□9.39	1905.06□123.33	1060.50□58.42	804.58□30.22
	Midrib	1.14±0.05	2.06±0.11	348.38□5.15	1392.86□86.57	775.88□31.52	790.52□49.46

Table 2. RAPD primer with corresponding bands scored, their size range, number of monomorphic and polymorphic bands, polymorphism and number of per variety in five brinjal varieties.

Primer Codes	Size range (bp)	Total No. of bands	No. of Mono-morphic bands	No. of Poly-morphic bands	Polymor-phism (%)	No. of bands per variety
OPW-04	900-80	10	7	3	30%	2
OPW-10	600-1000	20	11	9	45%	4
OPW-09	500-1000	27	15	12	44.44%	5.4
OPW-16	500-1000	24	12	12	50%	4.8
Total		81	45	36	44.44%	
Average		20.25	11.25	9		

Transverse section of leaf midrib

The transverse section of leaf showed a single layer uniseriate epidermis covered by a slightly thicker cuticle on the adaxial surface. The epidermal cells had an angular shape, and the adaxial surface cells were larger than the abaxial cells. The palisade parenchyma consisted of a single layer of elongated cells and the spongy parenchyma showed 4-5 layers of cells with varying shapes and noticeable intercellular spaces. The midribs of the five varieties were observed with two vascular traces and had bi-collateral vascular system. It was observed that the departures of the rib-bundle wings are towards the position of the open vascular system.

Table 3. Summary of linkage distances values for different cultivar pairs of Brinjal varieties.

	Golmakra	Debjhuri	Golapi F1	Kanta	China
Golmakra	0	0.7778	0.7778	0.6667	0.4444
Debjhuri	0.2513	0	0.7778	0.8889	0.6667
Golapi F1	0.2513	0.2513	0	0.8889	0.6667
Kanta	0.4055	0.1178	0.1178	0	0.7778
China	0.8109	0.4055	0.4055	0.2513	0

The bicollateral vascular bundle was immersed in the mesophyll of the four species studied, with intraaxillary phloem. Although intraaxillary phloem, which is unidirectional and differentiated only intok phloem elements in the centripetal direction, is reported in several other families, there are very few species that develop internal cambium at the pith margin.

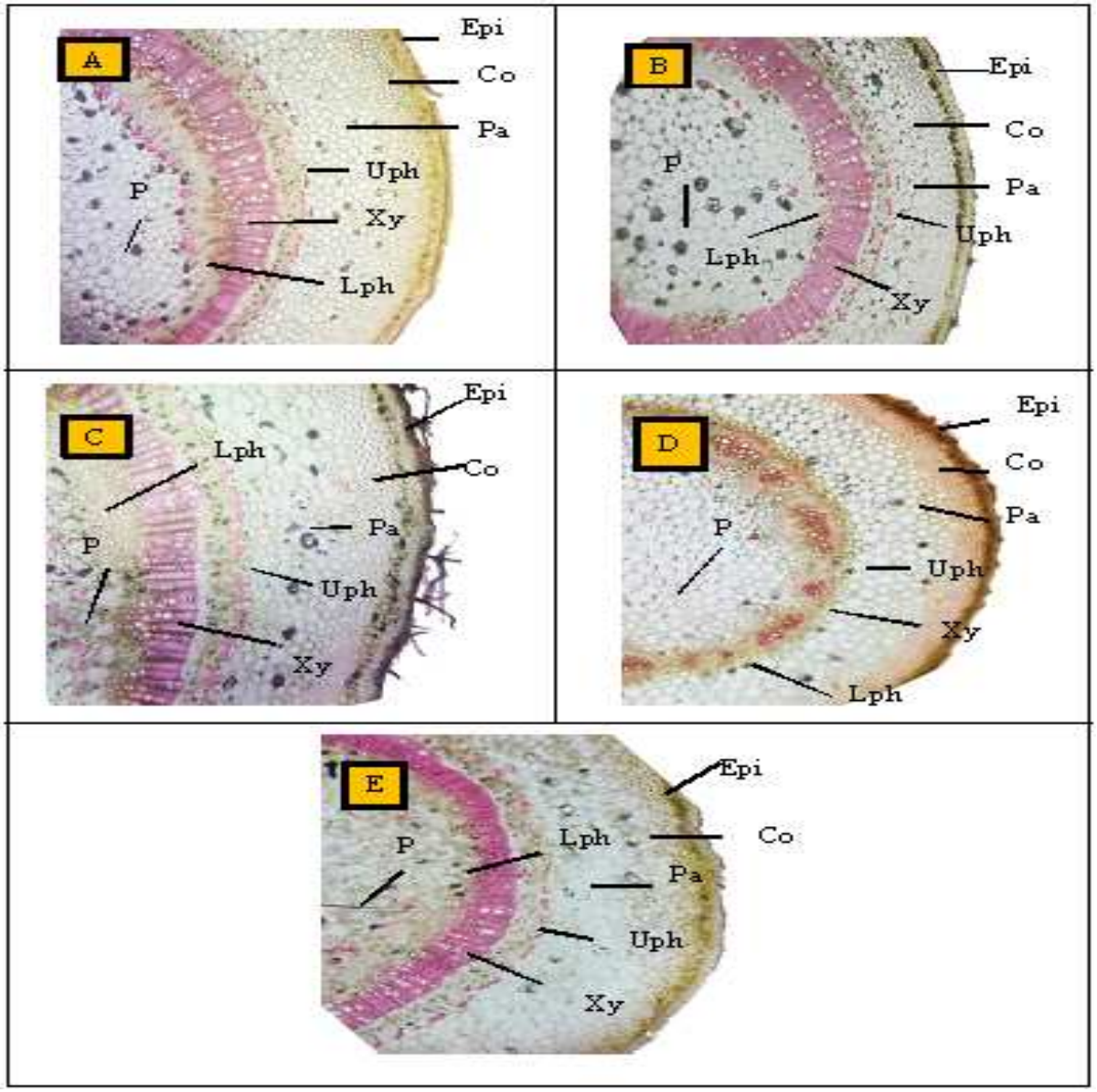

Fig. 1. Transverse section of stem (A= China, B= Golmakra, C=Debjhuri hajari , D=Golapi- F1, E= kanta begun) magnification of 100x, Epi= epidermis,Co=Colenchyma Pa= Parenchyma, Uph=Upper phloem, Xy= Xylem, Lph=Lower phloem, P=Pith.

Genetical studies

Number of bands

Four primers generated 81 bands from the five brinjal variety using the Thermal Cycler (Genius, Techne) and 1% agarose gel electrophoresis with size ranging from 500bp to 1000bp. Representative electrophoregrams according to primers OPW-04, OPW-10, OPW-09 and OPW-16, were shown in Figure 3, respectively. For four primers, the total number of bands 81 varied from 10 to 27. The primer OPW-09 amplified the highest number of bands 27 and the primer OPW-04 amplified the lowest number of bands. Out of the 81 bands, 45 bands were monomorphic bands and 36 bands were polymorphic bands. The primer OPW-16 produced the 12 polymorphic bands. Thus, it showed higher level of polymorphism 50%. The highest number of bands 5.4 per varieties was amplified from these primers.

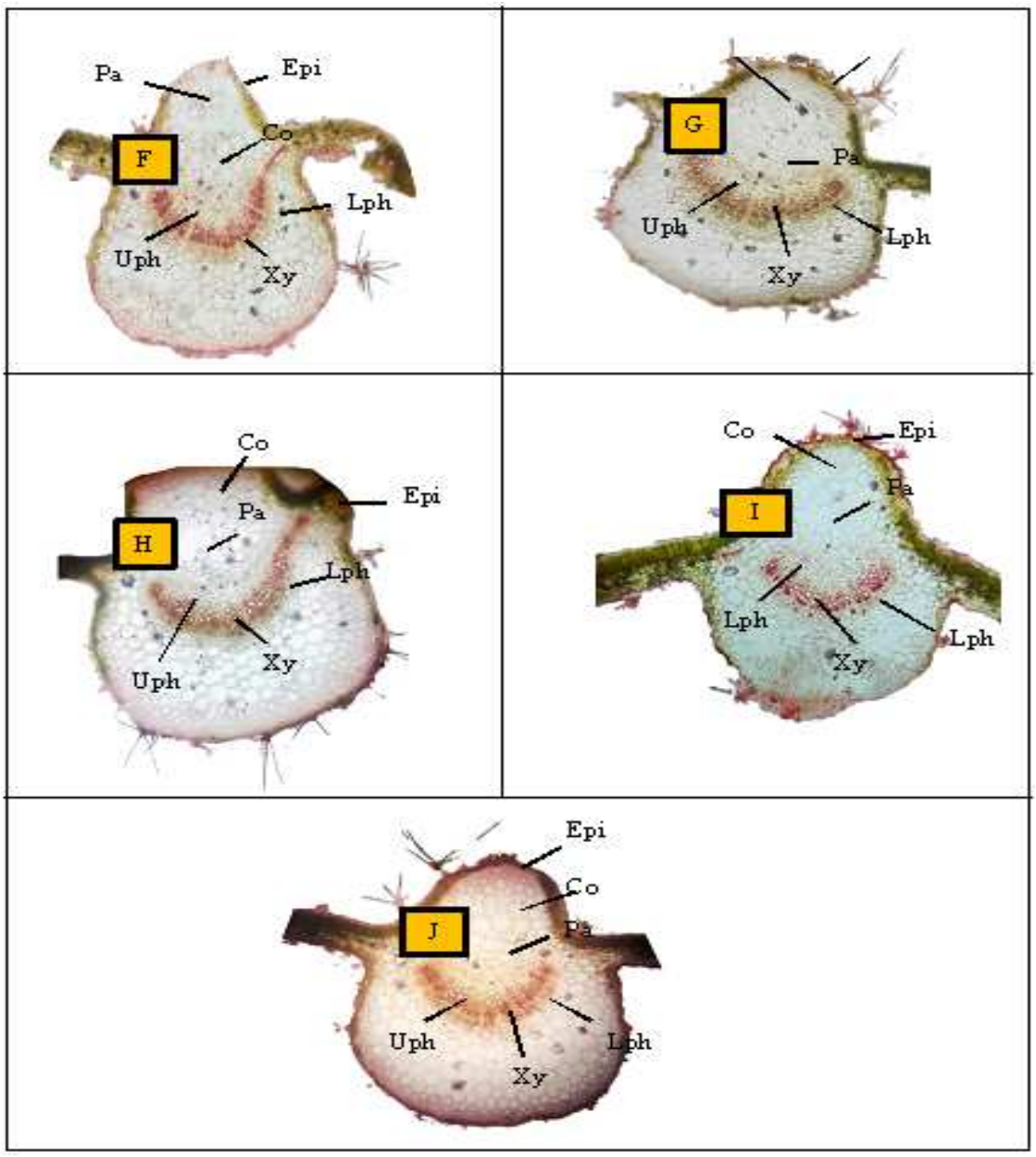

Fig. 2. Transverse section of midrib (F= China, G= Golmakra,H=Debjhuri hajari ,I=Golapi- F1, J= kanta begun) magnification of 100x, Epi= epidermis,Co=Colenchyma, Pa= Parenchyma, Uph=Upper phloem, Xy= Xylem, Lph=Lower phloem.

Genetic distances

The values of pair-wise comparisons of linkage distance analyzed by using computer software "Statistica" between strains were from combined data for the four primers, ranged 0.1178 to 0.8889. The highest linkage distance (0.8889) was found in brinjal variety Kanta begun, Debjhuri vs Golapi F1. The lowest linkage distance (0.1178) was found in variety Kanta begun vs debjhuri, Golapi F1 vs Kanta begun Pairs.

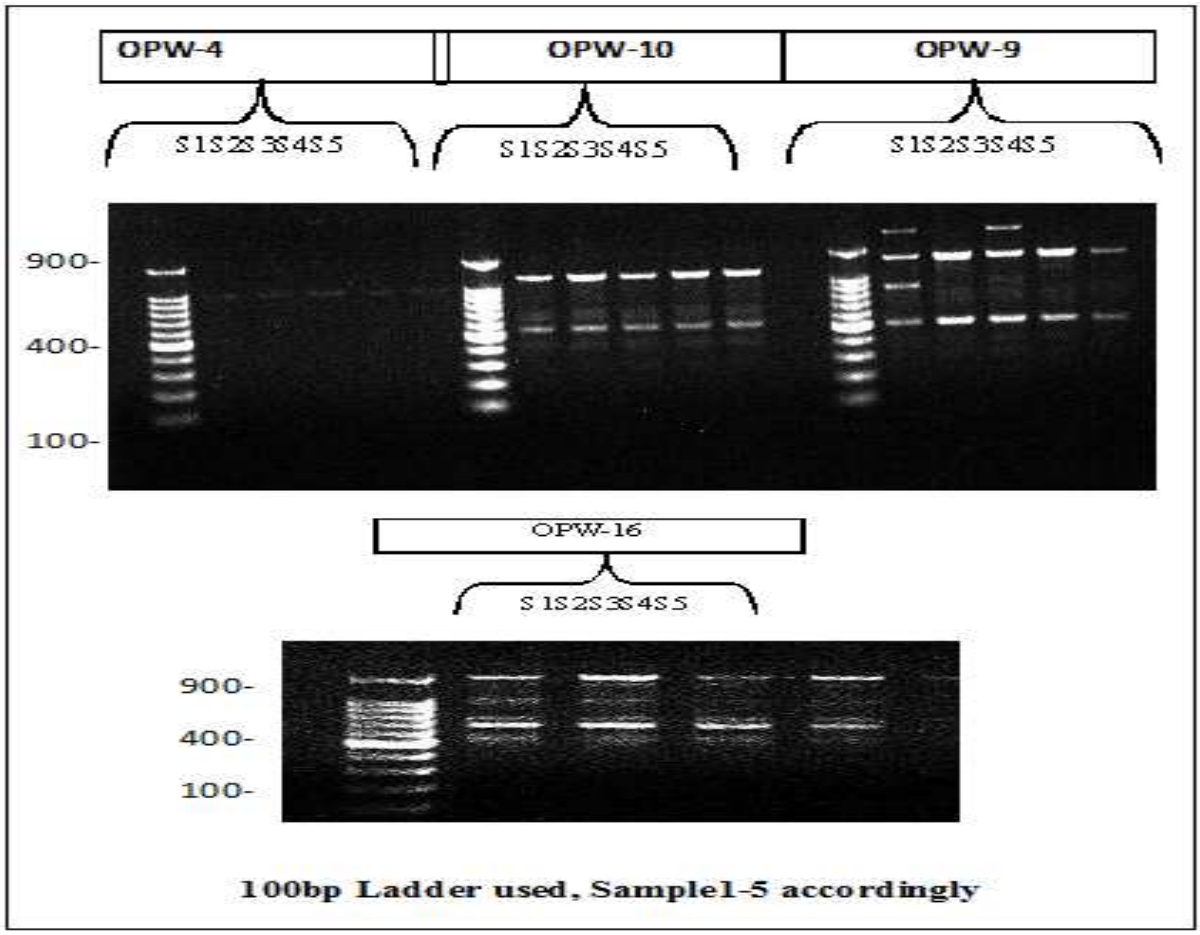

Fig. 3. RAPD profiles of OPW-04, OPW-10, OPW-09, OPW-16 primers generated from 5 different varieties (S_1= Golmakra, S_2= Debjhuri hajari, S_3=Golapi-F_1, S_4=Kanta begun, S_5=China).

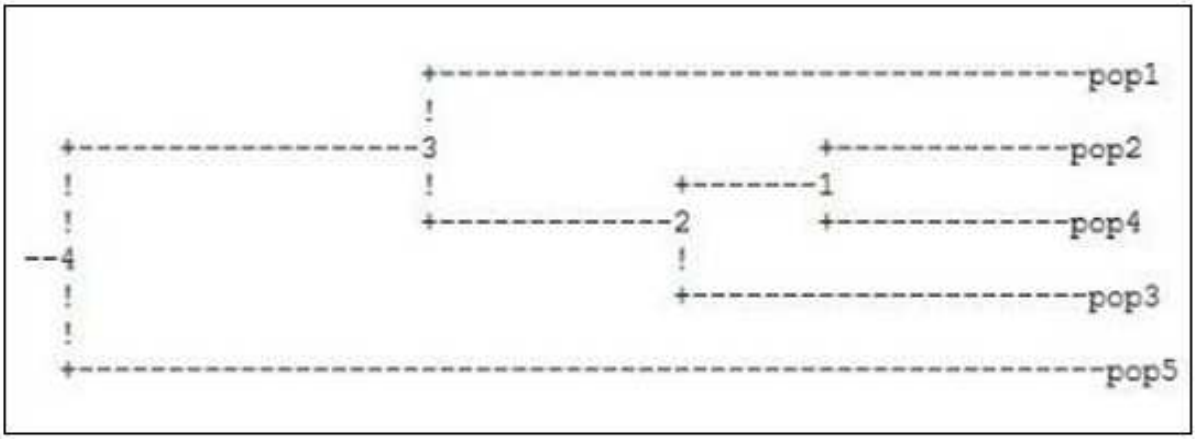

Fig. 4. Cluster Analysis by Unweighted pair group method of Arithmetic Means (UPGMA) of five brinjal varieties based on four RAPD markers (pop1=Golmakra, pop2=Debjhuri, pop3=Golapi -F_1, pop4=Kanta, pop5 =China).

Cluster analysis

Genetic relationship of five brinjal varieties based in RAPD data using unweighted pair group method with arithmetic mean (UPGMA) (Nei's, 1978). Genetic relationship among the brinjal varieties showed four major cluster (1, 2, 3, and 4) presented in the Figure 4. Golmakra was closely related to China. In the dendrogram, it was indicated that Debjhuri, Golapi F1 and Kanta begun was related. It was also indicated that China was superiorly different from other cultivated varieties.

Conclusions

Brinjal is a significant vegetable plant, and knowledge of genetics and the structure of the genome using molecular markers is extremely valuable for plant breeding and genetic research. To ascertain the genetic diversity and relationships among the Brinjal, RAPD markers would be quite helpful. It can produce polymorphism that distinguishes between genotypes that are closely related.

References

Ali Z, Xu ZL, Zhang DY, He XL, Bahadur S, Yi JX. 2011. Molecular diversity analysis of eggplant (*Solanum melongena* L.) genetic resources. Genetics and Molecular Research **10(2),** 1141-1155.

Afzal MA, Haque MM, Shanmugasundaram S. 2004. Random Amplified Polymorphic DNA (RAPD) analysis of selected mungbean (Vigna radiata L. Wilczek cultivars. Asian Journal of Plant Sciences **3(1),** 20-24.

Barrell PJ, Meiyalaghan S, Jac.obs JME, Conner AJ. 2013. Applications of biotechnology and genomics in potato improvement. Plant Biotechnology Journal **11,** 907-920.

BBS. 2012. Statistics Year Book of Bangladesh, Bangladesh Bureau of Statistics, Ministry of Planning, Government of the people's Republic of Bangladesh, Dhaka.

Collonnier C, Fock I, Mariska I, Servaes A, Vedel F, Siljak-Yakovlev S, Souvannavong V. Sihachakr D. 2003. GISH confirmation of somatic hybrids between *Solanum melongena* L. and S. torvum: assessment of resistance to both fungal and bacterial wilts. Plan Physiol. Biochem **41(5),** 459-470.

Cipriani G, Di Bella R, Testolin R. 1996. Screening RAPD primers for molecular taxonomy and cultivar fingerprinting I the genus Actinidia. Euphytica **90(2),** 169-174.

Gleddie S, Keller W, Setterfield G. 1986. Eggplant.In: Evans, D.A.,Sharp W.R. (Eds.), Handbook of Plant Cell Culture, Techniques for Propagation and Breeding. MacMillan, New York, **3,** 500-511 p.

Harish DK, Agasimani AK, Imamsaheb SJ, Patil Satish S. 2011. Growth and yield parameters in brinjal as influenced by organic nutrient management and plant protection conditions. Agricultural Science Research Journal **2(2),** 221-225.

Hinata H. 1986. Eggplant (*Solanum melongena* L.).In: Bajaj, Y.P.S. (Ed.), Biotechnology in Agriculture and Forestry, Crop I. Springer, Berlin, **2,** 363-370 p.

Jones N, Ougham H, Thomas H. 1997. Genome Mapping, molecular markers and Marker assisted selection in crop plants. New Phytologist **137,** 165-177.

Khan MA, Rather TH, Dar MA, Kudesia R. 2013. Genetic diversity analysis of brinjal (*Solanum melongena* L.) accessions using RAPD markers. International Journal of Current Research **5(5),** 1223-1223.

Kumar G, Meena BL, Kar R, Tiwari 2008. Morphological diversity inbbrinjal (*Solanum melongena* L.) germplasm accessions. Plant Genetic Resources **6(3),** 232-236.

Rafalski JA, Tingey SV. 1993. Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. Trends Genet **9,** 275-279.

Rashid M. 1993. Sabjee Bighan. Bangla Academy, Dhaka 137-14 p.

Rashid M. 1993. Sabjee Bighan.Bangla Academy, Dhaka, 137-144 p.

Spooner DM, Knapp S. 2013. Solanum stipuloideum Rusby, the correct name for Solanum circaeifolium Bitter. American Journal of Potato Research **90(4),** 301305.

Sing A, Borthakur A, Deka PC. 2001. Biotechnology of horticultural crops.2nd Eds. Vol.1.Naya Prakash.206 Bidhan Sarani, Kolkata, India p 35-39.

Sarathbabu B, Varaprasad KS, Charaabarty SK, Sivaraj N. 1999. Status of resistant germplasm in eggplant (*Solanum melongena* L.) and wild *Solanum* species. Indian Journal of Plant Genetic Resources **12,** 56-64.

Shaha MG. 1989. Combining ability estiamates and hybrid vigour in eggplant (*Solanum melongena* L.).M.Sc.(Ag) Thesis, Department of Horticulture, BAU, Mymensingh.

Sihachakr D, Daunay MC, Serraf I, Chaput MH, Mussio I, Haricourt R, Rotino L, Ducreux G. 1994. Somatic hybridization of eggplant (*Solanum melongena* L.) with its close and wild relatives. In: Bajaj YPS (ed.) Biotechnology in Agriculture and Forestry: Somatic Hybridization in Crop Improvement, Springer, Berlin, 255-278 p.

Sneath PHA, Sokal RR. 1973. Numerical taxonomy: the principles and practice of numerical classification. San Francisco: Freeman. 573 p.

Thompson HC. 1951. Vegetable crops, McGraw Hill Book.Co. New York. 368-370 p.

Thompson HC, Kelly WC. 1957. Vegetable crops, McGraw Hill Book. Co. New York. 500-505 p.

Tosti N, Negri V. 2002. Effiency of three PCR-based markers in assessing genetic variation among cowpea (vigna ungiculata spp unguiculata) landraces. Genome **45,** 268-275.

Williams JGK, Hanafey MK, Rafalski JA, Tingey SV. 1990. Genetic analysis using random amplified polymorphic DNA markers.Methods lEnzymology **1218,** 704-740.