Electronic Journal of Plant Breeding, 5(4): 722-728 (Sep 2014)
ISSN 0975-928X

Research Article

Molecular diversity of brinjal (*Solanum melongena* L. and *S. aethiopicum* L.) genotypes revealed by SSR markers

Abdul Majid Ansari[1,*] and Y. V. Singh
Department of Vegetable Science, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar - 263 145 (Uttarakhand), India
[1]ZRS, Chianki (Birsa Agricultural University, Ranchi), Palamau-822102 (Jharkhand), India
Email: majid.gbp@gmail.com

(Received: 23 July 2014; Accepted: 08 Sep 2014)

Abstract
In the present study, simple sequence repeat (SSR) markers were used to study the genetic diversity among 14 genotypes of brinjal. A total of 14 polymorphic SSR primer pairs were used. Amplification of genomic DNA of 14 genotypes yielded 50 fragments, of which 43 were polymorphic. A clear cut differentiation was exhibited among the genotypes. The range of similarity coefficient varied from 17.8% [between *S. aethiopicum* L. (2n=2x=24) and Pant Rituraj (*S. melongena* L., 2n=2x=24)] to 94.1% [between PB-71 and NDB-1] followed by 88.9% [between SMB-115 and KS-331] and 88.6% [between BARI and PB-67]. SAHN cluster analysis using UPGMA method separated the genotypes into six cluster groups. *Solanum aethiopicum* and PB-67 were positioned as single genotype in separate groups i.e., cluster-I & II, SMB-115 and KS-331 in cluster-III, BARI, PB-66 and Pant Rituraj in cluster-IV, WB-1, PB-4, PB-70 and LC-7 in cluster-V and PB-71, Pant Samrat and NDB-1 in cluster-VI. Morphological characters viz., shape, size and peel colour of brinjal fruits and plant type showed a positive relationship with the DNA based molecular analysis through SSR markers.

Keywords
Brinjal, *Solanum, melongena, aethiopicum,* SSR markers, genetic diversity.

Introduction

Brinjal (*Solanum melongena* L. 2n=2x=24) is known as eggplant in United States and *aubergine* in France and England. It is an important vegetable crop of the *Solanaceae* family. In India, it is popularly known as *baigan, bhanta, badankai, vangi,* etc., where it was domesticated very long ago and its wide range of diversity exists. Now, India is considered as center of origin and diversity of brinjal (Vavilov, 1951; Isshiki *et al*., 1994). It is also a popular vegetable crop in most of the Asian and European countries. Other species of the genus *Solanum* are also important for medicinal as well as breeding purposes. In Africa, *S. aethiopicum* L. (2n=2x=24) group *gilo* and *S. anguri* are used for the treatment of many diseases and many other *Solanum* species are also used for medicinal purposes (Bukenya and Carasco, 1999).

The Polymerase Chain Reaction **(**PCR) based molecular markers such as RAPD, SSR and ISSR have successfully been utilized to assess the genetic diversity among the genotypes of several crop plants. Out of these, SSR are highly informative and locus specific genetic markers which are co-dominant in nature with high information content (Danin-Poleg *et al*., 2000). SSR has facilitated the studies of genetic diversity (Plaschke *et al*., 1995), gene mapping (Pestsova *et al*., 2002) and testing of authenticity of genetic stocks (Pestsova *et al*., 2002). It is typically multi-allelic marker (Matsuoka *et al*., 2002) with heterozygosity values much higher than those of RFLPs (McCouch, 1997). Nunome *et al*. (2003) were the first to use SSRs markers for studying molecular diversity among the genotypes of brinjal. Hence, the present investigation was carried out with microsatellite (SSR) markers for analysis of genetic diversity of 13 genotypes of *S. melongena* and with one genotype of *S. aethiopicum.*

Material and methods

PCR based molecular diversity work was carried out in the PG Laboratory of the Department of Vegetable Science and Plant Molecular Biology Laboratory of the Department of Genetics and Plant Breeding, College of Agriculture, GBPUA&T, Pantnagar, Uttarakhand, India.

Plant materials: The materials used in the investigation comprised of 13 accessions of *Solanum melongena* and one accession of *Solanum aethiopicum* have been depicted in Table 1 along with geographical locations. Each accession was sown in pots and leaf sample from 2-3 week old seedlings were collected for genomic DNA isolation.

Genomic DNA Extraction and quantification: Freshly collected leaf sample of each accession was ground to fine powder in mortar and pestle using liquid nitrogen and immediately it was processed using the Plant Genomic DNA isolation kit (HiMedia Laboratories Pvt. Ltd, India). The kit was especially designed for plant genomic DNA

isolation and based on Cetyl Trimethyl Ammonium Bromide (CTAB) method. The RNA content in genomic DNA isolated using the kit was removed using RNase A. Quantity and quality of the genomic DNA was determined using dual beam spectrophotometer (Systronics, India) and Agarose gel electrophoresis (Sambrook and Russel, 2001).

DNA Markers: Polymerase chain reaction (PCR) based marker SSR was used to quantify the genetic diversity of the *aethiopicum* and *melongena* species. A total of 14 SSRs were used in the investigation which have been depicted in Table 2.

PCR Amplification: PCR amplification was performed in a volume of 25 µl. Each 25µl reaction mixture consisted of 100 ηg of DNA, 200 µM dNTPs, 1U of Taq polymerase, 1X Taq buffer and 1.2 µM primer of SSR each of forward and reverse primers were used. Amplication reaction was performed with initial denaturation at 94 ºC for 5 min followed by 35 cycles of denaturation at 94 ºC and 30 second, annealing of primers for 1 min at 45-60 ºC and polymerization for 1 min at 72 ºC. One cycle of final extension was maintained for 5 min at 72 ºC. Amplification reaction was performed in dual block DNA engine (Bio-Rad, USA). PCR amplified DNA fragments were resolved using 3% Agarose gel for electrophoresis to resolve the fragments generated using SSR primers. The gels were stained with Ethidium bromide (0.5mg/ml) and documented using Gel Documentation system (Alpha Imager EC).

Scoring of gel and analysis of data: Each genotype was scored as presence (1) or absence (0) of respective allele against each primer. Pair wise Jaccard's similarity coefficients (1908) were calculated to determine the similarity of *S. melongena* and *S. aethiopicum* genotypes. Unweighted pair group method with arithmetic average (UPGMA) based dendrogram was generated to determine marker based genetic relationship amongst the 14 genotypes. The data were analyzed using the NTSYS-pc software (Rohlf, 2000).

Results and discussion

Fourteen SSR primers exhibited a total of 50 bands in the 14 genotypes. The number of alleles per locus varied from one (EEMS17 and EM145) to seven (EM140). Six primers viz. EMB01L13 for SMB-115 (L_{12}) and KS-331 (L_{13}), EEMS28 for *S. aethiopicum* (L_1), PB-66 (L_3) and PB-4 (L_{11}), EEMS48 for *S. aethiopicum* (L_1) and WB-1 (L_5), EEMS49 for *S. aethiopicum* (L_1) and BARI (L_2), EM119 for *S. aethiopicum* (L_1), SMB-115 (L_{12}) and LC-7 (L_{14}) and EM140 for PB-4 (L_{11}) revealed unique locus for these genotypes showing the informativeness of the marker (Table 3). Out of 50 bands, 43 bands were polymorphic (table 3). Amplified fragments ranged from 80 bp (emf21H22 and EEMS48) to 380 bp (EMB01H20, EM119 and EM145) amongst different genotypes. The average number of bands per primer was 3.57 while in case of polymorphic bands only 3.07 bands per primer were obtained (Table 4).

Similarity coefficient: Pair wise Jaccard's similarity coefficients using 14 SSR markers were estimated for all the genotypes. The range of the coefficient varied from 17.8% between *S. aethiopicum* and Pant Rituraj to 94.1% between PB-71 and NDB-1 followed by 88.9% between L12 and L13 and 88.6% between BARI and PB-67. The values of similarity coefficients are given in Table 4.

Cluster analysis: The phylogenetic tree was constructed through SAHN cluster analysis (Fig. 1) using UPGMA method. SAHN cluster analysis using UPGMA method separated the genotypes into six cluster groups (Table 5). *S. aethiopicum* (2n=2x=24) was positioned in a separate group-I through SAHN cluster analysis. It is a small light green round fruited genotype which turn scarlet red while ripening. Long light green fruited genotype PB-67 was also positioned in other separate group-II which was morphologically quite different with other commercial genotypes of brinjal, having soft fruit with less seeds and light green with bottle gourd like appearance at tender stage. Two cluster bearing genotypes *viz*., SMB-115 and KS-331 were positioned in the same group-III, dark purple fruited genotypes *viz*., BARI, PB-66 and Pant Rituraj were in group-IV, oblong fruited genotypes *viz*., WB-1, PB-4, PB-70 and LC-7 were in the group-V whereas, erect plant type *viz*., PB-71, Pant Samrat and NDB-1 were in the last group-VI. It showed that there is a positive relationship between morphological characters based diversity and DNA based molecular diversity among the genotypes of *S. aethiopicum* and *S. melongena*.

Primer informativeness (SSR): Out of fourteen SSR primers, EM119 amplified six loci with four polymorphic and three unique alleles to differentiate three different genotypes. Nine primers showed 100% polymorphism, whereas two primers *viz*., EEMS 15 and EEMS 17 showed monomorphic bands. Based on % polymorphism and unique band patterns six primers *viz*. EMB01L13, EEMS28, EEMS48, EEMS49, EM119 and EM140 could be used for identification of respective genotype.

The evaluation of germplasm and assigning them in values is very much important for a breeder to plan an effective hybridization programme to produce new genotypes for higher productivity, adaptability and quality. Molecular markers are now considered

the most effective tool today to characterizing and quantifying allelic diversity in a set of germplasm. SSRs have been used in determining molecular diversity (Nunome *et al.*, 2003; Stagel *et al.*, 2008; Nunome *et al.*, 2009). In this investigation we used 14 SSR markers to analyz the diversity among *Solanum* genotypes.

SSR marker discriminated *S. aethiopicum* from 13 genotypes of *S. melongena* and formed two major groups with one genotype of *S. aethiopicum* was differentiated with second group with 13 genotypes of *S. melongena* by similarity coefficient of 0.24. The marker analysis therefore indicates that *S. aethiopicum* had wide molecular diversity with *S. melongena*. High diversity is further supported by the homologous chromosome complement of *S. melongena* and *S. aethiopicum* revealed by moderate bivalent formation coupled with higher percentage of multivalent in the F_1 plus the occurrence of univalent (Roxas *et al.*, 1995).

BARI, one of the genotype of *melongena* group included in the study is an extra-long fruited variety (up to 45 cm long fruit) is popular in Bangladesh. Similarly, genotypes WB-1, SMB-115 and KS-331 as well as NDB-1 are the cultivars belonging to West Bengal, Orissa and Uttar Pradesh state of India, respectively. The remaining genotypes have been developed at Pantnagar, Uttarakhand in India which is situated in the foothills and *tarai* belt of the great *Himalayas.* Distribution of genotypes from different geographical region did not show clear clustering pattern in the SSR based genetic diversity analysis, which indicated that the association between genetic similarity and geographical distance is less significant. Gene flow intentional or natural in the past might be a probable reason for such a distribution pattern based on this marker data. Yee *et al.* (1999) and Li *et al.* (2013) noted similar observation while analyzing Azuki bean and Ruthenia Medic. Similar results also reported by Demir *et al.* (2010) that carried out molecular characterization of brinjal genotypes collected from different geographical regions of Turkey using SSR markers. They found that the number of alleles per microsatellite locus ranged from 2 to 10, with a total of 24 alleles with the amplification of five SSR loci. The greatest number of alleles was found at the *emf21H22* locus (10 alleles); followed by *emh11O01* and *emf21C11* as five and four alleles, respectively.

Genotypes possessed most distinct DNA profiles are expected to contain the greatest number of unique/novel alleles. Such genotypes are likely to serve as source to uncover the largest number of unique and agronomically useful alleles (Souframanien and Gopalakrishna, 2004). In this investigation, *S. aethiopicum* exhibited distinct DNA profile with 11 unique/novel alleles with SSR marker. These alleles may be agronomically important therefore, it requires further investigation to uncover its potential of novelty. The unique/novel alleles identified in *S. melongena* also prove to be associated with desirable agronomic traits may serve as important in the improvement programme. With the help of this study we successfully determined the diversity between the two species of *Solanum* that may be utilized in the breeding and pre-breeding programme of brinjal. Amplification of genomic DNA yielded 50 fragments, of which 43 were polymorphic. SAHN cluster analysis using UPGMA method separated the genotypes into six cluster groups. Morphological characters viz., shape, size and peel colour of brinjal fruits and plant type showed a positive relationship with the DNA based molecular analysis through SSR markers.

References

Bukenya, Z.R. and Carasco, J.F. 1999. Ethno-botanical aspects of *Solanum* L. (*Solanaceae*) in Uganda. *In*: Solanaceae *IV. Advances in Biology and Utilization* (EdsNei, M. M., Symons, D. E., Lester, R. N. and Jessop, J. P.), Royal Botanic Gardens, Kew, UK. pp. 345-360.

Danin-Poleg, Y., Reis, N., Baudracco-Arnas, S., Pitrat, M., Staub, J.E., Oliver, M., Arus, P., de Vicente, C.M. and Katzir, N. 2000. Simple sequence repeats in *Cucumis*, mapping and map merging. *Genome*, **43**: 963-974.

Demir, K., Bakir, M., Sarikamis, G. and Acunalp, A. 2010. Genetic diversity of eggplant (*Solanum melongena* L.) germplasm from Turkey assessed by SSR and RAPD markers. *Genet.Mole. Res.*, **9**(3): 1568-1576.

Isshiki, S., Okuba, H. and Fujieda, K. 1994. Phylogeny of eggplant and related *Solanum* species constructed by allozyme variation. *Scientia Horti.*, **59**: 171-176.

Jaccard, P. 1908. Nouvelles researches sur la distribution florale. *Bul. Soc. Vaud. Sci. Nat*., **44**: 223-270.

Li, H., Li, Z., Cai, L., Shi, W., Fu Mi and Shi, F. 2013. Analysis of genetic diversity of Ruthenia Medic (*Medicago ruthenica* L.) in Inner Mongolia using ISSR and SSR markers. *Genet. Resour. Crop Evol.*, **60**: 1687-1694.

Matsuoka, Y., Mitchell, S.E., Kresovich, S., Goodman, M. and Doebly, J. 2002. Microsatellite in *Zea*; variability, pattern of mutations, and use for evolutionary studies. *Theor. Appl. Genet.*, **104**: 436-450.

McCouch, S.R. 1997. Comparative evaluation of within-cultivar variation of rice (*Oryza sativa* L.) using microsatellite and RFLP markers. *Genome*, **40**: 370–378.

Nunome, T., Suwabe, K., Iketani, H. and Hirai, M. 2003. Identification and characterization of microsatellites in eggplant. *Plant Breed.*, **122:** 256-262.

Nunome, T., Negoro, S., Kono, I., Kanamoti, H., Miyatake, K., Yamaguchi, H., Ohyama, A. and Fukuoka, H. 2009. Development of SSR

markers derived from SSR-enriched genomic library of eggplant (*Solanum melongena* L.). *Theor. Appl. Genet.*, **119**: 1143-1153.

Pestsova, E., Ganal, M.W. and Roder, M.S. 2002. Isolation and mapping of microsatellite markers specified for the genome of bread wheat. *Genome*, **43**: 689-697.

Plaschke, J., Ganal, M.W. and Roder, M.S. 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. *Theor. Appl. Genet.*, **91**: 1001-1007.

Rohlf, F.J. 2000. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Version 2.1 Exceter Software, New York, USA.

Roxas, D.M.R., Vina dela, A.C. and Ramirez, D.A. 1995. Cytogenetics of *Solanum melongena* L., *Solanum aethiopicum* and their F_1 hybrid. *Philipp. J. Crop Sci*., **20**: 129-140.

Sambrook, J. and Russel, D.W. 2001. Molecular cloning: A laboratory manual. *Cold Spring Harbor Laboratory Press*, Cold Spring Harbor, New York.

Souframanien, J. and Gopalakrishna, T. 2004. A comparative analysis of genetic diversity in black gram genotypes using RAPD and ISSR markers. *Theor. Appl. Genet*., **109**: 1687-1693.

Stagel, A., Portis, E., Toppino, L. and Rotino, G.L. 2008. Gene-based microsatellite development for mapping and phylogeny studies in eggplant. *BMC Genomics*, **9**: 357.

Vavilov, N.I. 1951. Phytogeographical basis of plant breeding. The origin, variation, immunity and breeding of cultivated plants (K. J. Choster translator). *Chranica Botanica*, **13**: 366.

Yee, E., Kidwell, K.K., Sills, G.R. and Lumpkin, T.A. 1999. Diversity among selected *Vigna angularis* (Azuki) accession on the basis of RAPD and AFLP markers. *Crop Sci.,* **39**: 268-275.

Table 1. List of genotypes utilized in the experiment

S. No.	Genotypes	Source	Given code
1	*Solanum aethiopicum*	Pantnagar	L_1
2	BARI	Bangladesh	L_2
3	PB-66	Pantnagar	L_3
4	Pant Rituraj (PR)	Pantnagar	L_4
5	WB-1	Koochbihar (West Bengal)	L_5
6	PB-67(PB-6)	Pantnagar	L_6
7	PB-71	Pantnagar	L_7
8	Pant Samrat (PS)	Pantnagar	L_8
9	NDB-1	NDUAT, Faizabad	L_9
10	PB-70	Pantnagar	L_{10}
11	PB-4	Pantnagar	L_{11}
12	SMB-115	Cuttack	L_{12}
13	KS-331	Kalyanpur (UP)	L_{13}
14	LC-7	Pantnagar	L_{14}

Table 2. Sequences of the SSR Primers

S. No.	Source Name	Forward primer Sequence (5′- 3′)	Reverse primer Sequence (5′- 3′
01	*emh*11O01	GATGTGTCGATGAGATTTTGGTCA	TAGCTACGTTGGTTTGGTGCTGAA
02	EMB01L13	TCAAAAGACTTGAAACCCGATGGT	GTTTATCAGGTTTTTGATCACCGGACA
03	EMB01H20	TCTTGTTCCCAGTCTATCGCTAATCA	ATCCGAATTTAGTCGGGCTTCAAT
04	*emf*21C11	TGGTTGGAGCCATGATTACTTGAA	ATGCTACCTATCAAACAGGCGGAA
05	*emf*21H22	CACAAGATGAACAAGACTAAGGAGTGC	CTTCTTCAACCTGTCTTTAGCCCA
06	EEMS15	GGGACAAATCTGACCTTTGG	CTGGTGGCAAATTCTTCGAT
07	EEMS17	TGACATGTAGCTGGGCAGAG	TGGAGTGTGCATCCCAAATA
08	EEMS28	GACGATGACGACGACGATAA	TGGACTCACAACTCAGCCAG
09	EEMS48	CAATGCAAACAATTATCATTTCG	TCGATGTTGTTGTCGTCGTT
10	EEMS49	TGAAATTGATCAATACCTATAAATTTAG	GAAAGCCAGGATAGCATTCG
11	EM119	CCCCACCCCATTTGTGTTATGTT	ACCCGAGAGCTATGGAGTGTTCTG
12	EM140	CCAAAACAATTTCCAGTGACTGTGC	GACCAGAATGCCCCTCAAATTAAA
13	EM145	CAGTGCTACATAAATTGAGACAAGAGG	GGAGGTACAACGATTTTCATATGGT
14	EM155	CAAAAGATAAAAAGCTGCCGGATG	CATGCGTGAGTTTTGGAGAGAGAG

Table 3. Analysis of SSR marker

S. No.	Primers Code	% Polymorphism	Number of bands			Unique bands	
			Total bands	Monomorphic Bands	Polymorphic Bands	‘a’	‘m’
1	emh11O01	100.00	2	0	2	-	-
2	EMB01L13	100.00	2	0	2	-	1
3	EMB01H20	100.00	5	0	5	1	-
4	emf21C11	100.00	3	0	3	1	-
5	emf21H22	100.00	3	0	3	2	-
6	EEMS15	00.00	2	2	0	-	-
7	EEMS17	00.00	1	1	0	-	-
8	EEMS28	100.00	6	0	6	1	1
9	EEMS48	100.00	6	0	6	1	1
10	EEMS49	100.00	2	0	2	1	1
11	EM119	67.00	6	2	4	3	1
12	EM140	86.00	7	1	6	-	1
13	EM145	100.00	1	0	1	-	-
14	EM155	75.00	4	1	3	1	-
Mean/ Total		**80.57**	**50**	**7**	**43**	**11**	**6**

‘a’ for *S. aethiopicum* and ‘m’ for *S. melongena*

Table 4. Estimated Jaccard's similarity coefficient of 14 germplasm of brinjal using SSR data

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14
L1	1													
L2	0.2	1												
L3	0.244	0.886	1											
L4	0.178	0.853	0.857	1										
L5	0.262	0.75	0.806	0.722	1									
L6	0.244	0.694	0.75	0.714	0.765	1								
L7	0.222	0.806	0.811	0.829	0.778	0.824	1							
L8	0.233	0.8	0.857	0.824	0.824	0.765	0.829	1						
L9	0.25	0.806	0.861	0.829	0.829	0.824	0.941	0.882	1					
L10	0.25	0.667	0.676	0.688	0.742	0.733	0.697	0.688	0.697	1				
L11	0.268	0.722	0.778	0.743	0.794	0.735	0.75	0.794	0.75	0.767	1			
L12	0.244	0.784	0.789	0.806	0.711	0.703	0.861	0.757	0.811	0.676	0.73	1		
L13	0.244	0.784	0.838	0.757	0.757	0.8	0.861	0.857	0.861	0.676	0.73	0.889	1	
L14	0.25	0.667	0.676	0.788	0.788	0.727	0.794	0.735	0.794	0.759	0.758	0.771	0.676	1

Note: 1=*S. aethiopicum, 2*= BARI, 3=PB-66, 4=PR, 5=WB-1, 6=PB-67, 7=PB-71, 8=PS, 9=NDB-1, 10=PB-70, 11=PB-4, 12=SMB- 115, 13=KS-331, 14=LC

Table 5. Clustering group at 80 % similarity level based on SSR using UPGMA dendrogram

Cluster group	No. of genotypes	Name of genotypes
I	1	*S. aethiopicum*
II	1	PB-67 (long green fruits)
III	2	SMB-115 and KS-331(both cluster bearing)
IV	3	BARI, PB-66 and PR (all three purple fruits)
V	4	WB-1, PB-4, PB-70 and LC-7 (all four oblong fruits)
VI	3	PB-71, PS and NDB-1 (all three erect plant type)

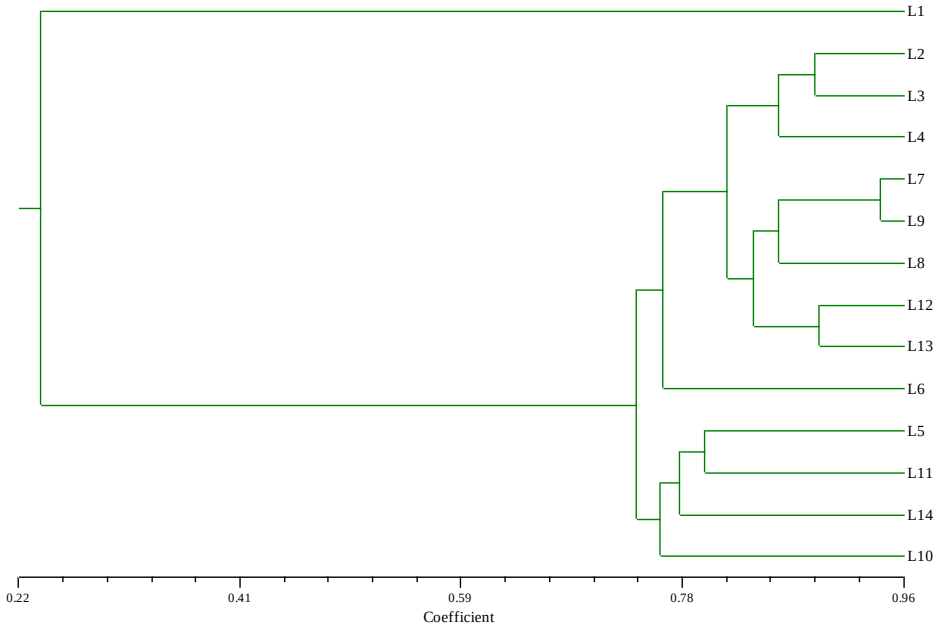

Figure 1. UPGMA dendrogram showing clustering of 14 genotypes of brinjal based on 14 SSR primers.
1=*S. aethiopicum*, *2*=BARI, 3=PB-66, 4=PR, 5=WB-1, 6=PB-67, 7=PB-71, 8=PS, 9=NDB-1, 10=PB-70, 11=PB-4, 12=SMB-115, 13=KS-331, 14=LC-7.