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SSR based molecular characterization of brinjal (*Solanum melongena*) genotypes for quantitative traits

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ABSTRACT

The study aimed to evaluate the genetic diversity of eight brinjal (*Solanum melongena* L.) genotypes including wild species and to develop a molecular profile using 14 SSR primers. The value of the similarity matrix ranged from 0.12–0.87. Dendrogram image is revealing the relationship among eight genotypes of brinjal. The super cluster is divided into two clusters A and B at Jaccard's similarity coefficient of 0.31. Cluster A again divide into two sub cluster A_1 and A_2 at Jaccard's similarity coefficient 0.45. A_1 contain three genotypes A_1i (SMB-115), A_1 ii (BARI), A_1 iii (Pant Rituraj) and A_2 contain only one genotype (Pusa Upkar) at Jaccard's similarity coefficient 0.88. Cluster B divided in two sub clusters B_1 and B_2 at Jaccard's similarity coefficient of 0.70. B_1 again divided into sub cluster B_1i (Pant Samrat), B_1ii (PB-6) and B_2 also divide into two sub cluster B_2 i (PB-101) and B_2ii (*S. gilo*) at Jaccard's similarity coefficient 0.88. Genotype 1 (SMB-115) and 6 (PB-101) were most diverse among all the eight genotypes indicating that the cross between genotype SMB-115 and genotype PB-101 has potential to give more heterotic hybrids due to their highly diverse nature. The results revealed that these microsatellite markers could be used as successful tool to differentiate the genetic makeup of the brinjal crop.

Keywords: Brinjal, Molecular Characterization, Quantitative Traits, SSR Marker, Wild relatives

Brinjal (*Solanum melongena* L.) (2n=2x=24) belongs to family solanaceae, with different names such as eggplant, aubergine and Guinea squash is one among the few cultivated species of solanaceae originating from the old world and moved to the new world (Daunay *et al.* 2001). The exotic species of cultivated *Solanum melongena,* viz. *Solanum indicum*, *Solanum gilo* and *Solanum incanum* are highly resistant to shoot and fruit borer; the most destructive pest of eggplant in India and other tropical and sub-tropical parts of the world. Wild relatives of eggplant are unique source of improvement for important traits, such as insect and disease resistance, drought tolerance and for quality traits, like a high content in bioactive phenolic acids (Gramazio *et al.* 2018). The crossability and hybridization studies of *S. melongena* and its related species have been generally inconclusive and the results are often contradictory (Nasrallah and Hopp 1963, Narsimha Rao 1979, Attavian *et al.* 1983). It is known as eggplant in North America, Australia and New Zealand, but British English uses the French word *aubergine*. Among the available different families, solanaceae is more useful as a leading source of vegetable. Vavilov (1928) was in the opinion of Indo-Burma as its center of origin however, the greatest diversity in its wild relatives is found in Africa (Knapp *et al.* 2013). Undoubtedly, the wild relatives in any crop are important reservoirs of useful genes and underexploited variation. Although eggplant is one among the vegetables with the highest concentrations in phenolic acids (Plazas *et al.* 2013), wild relatives can further contribute to a dramatic increase in these bioactive compounds which are highly beneficial for human health. Among all the available molecular markers, Simple Sequence Repeats (SSR) or microsatellites are most widely used and potentially the most informative molecular marker with the advantage of easy and low-cost detection by PCR. These are highly polymorphic even between closely related lines, stable, locus specific, co-dominant and also require a small amount of DNA (Roder *et al.* 1998). SSR markers detect more variability due to their multi-allelic nature (Roder *et al.* 1998) and are a useful marker system for marker-assisted selection.

MATERIAL AND METHODS

An experiment was conducted at Vegetable Research Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar during *kharif* 2017, 2018 and 2019 (June–December). This centre is topographically arranged

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at the attitude of 29.50° north, longitude of 79.30° east and at a height of 1129 feet (344 m) from the mean sea level. It falls under the moist subtropical zone, at the lower regions of the Shivalik hills of the Himalayas, in a narrow belt called *tarai*. The climate of the area is comprehensively sub-tropical however temperature ranges from 32–40°C during summer months. The rain starts from mid-June and remain continue up to September. Periodic light rains are normal during winter months too, frost mainly occurs from late December to February. Relative humidity is just about 80–90% from mid-June to end of February and afterward it consistently decreases to 50% by May and remains so till mid-June. Brinjal can be grown on all type of soil. Sandy loam soil is suitable for early crop while silt loam or clay loam soil is suitable for high yield. The soil of field at Vegetable Research Centre of Pantnagar was deep, fertile and sandy loam which is suitable for early crop and soil had *p*H of 7.2 which is also good for growth and development of plant.

MATERIALS AND METHODS

The experimental material in present investigation consisted of eight genotypes of brinjal collected from different sources. These genotypes are SMB 115 (Semi erect, lilac purple cluster bearing fruits suitable to round the year cultivation), BARI (Erect plant type, extra-long fruit having light purple colour, tolerant to Phomopsis blight), Pant Rituraj (Semi erect, round dark purple fruit, suitable to round the year cultivation), Pant Samrat (Erect, long purple fruits, cluster bearing, good combiners, field resistance to bacterial wilt, tolerant to Phomopsis blight and less affected by shoot and fruit borer), PB 6 (Semi erect, Green long fruit having dark purple flower), Pusa Upkar (Spreading plant type, round dark purple fruit, single solitary flower), PB101 (Erect, small, round and cluster bearing oblong white purple fruits which become complete white while full ripen) and *Solanum gilo* (Erect, small, round and cluster bearing small green fruits which become scarlet red while ripen, tolerant to shoot and fruit borer)

Extraction of genomic DNA: The genomic DNA was extracted for molecular characterization studies by using CTAB method of Doyle and Doyle (1990) with some modifications. Primers have the sequence complementary to the target DNA segment called template DNA to be synthesized (Table 1). SSR molecular markers were used to evaluate seedling.

DNA quality was ensured by electrophoresis of stock DNA in 0.8% agarose gel while quantification was done with spectrophotometer (Systronics PC Based Double Beam Spectrophotometer 2202). The DNA stocks were then diluted to a working concentration of 200 ng/µL and stored for further PCR amplification. Amplification of DNA was done in 13.8 µL reaction mixture consisting of 3 µL DNA template (200 ng/µL), 0.35 µL dNTPs mix (2.5 mM each), 0.25 µL Taq DNA polymerase (3U/µL), 1.5 µL reaction buffer with 15 mM MgCl2 (109), 1.5 µL of both forward and reverse primer (40 ng/µL) and 7.2 µL deionized water. The PCR amplification product was resolved in horizontal gel electrophoresis assembly using 3% agarose gel. The amplicons were visualized and photographed with UV light in gel documentation unit (Alpha Innotech Corporation, USA) after 75% of gel run.

RESULTS AND DISCCUSION

Fourteen SSR primers were used to screen seven *Solonum melongena* lines with one wild species. Binary data was subjected to NTSYS 2.01 software for generation of similarity matrix by SIMQUAL option. Pair-wise Jaccard's similarity coefficient was calculated (Table 2). The value of the similarity matrix ranged from 0.12–0.87. Dendrogram image is revealing the relationship among eight genotypes of brinjal (Fig 1). The super cluster is divided into two clusters A and B at Jaccard's similarity coefficient of 0.31. Cluster A again divide into two sub cluster $A_1$and A_2 at Jaccard's

Table 1 Primers used in the experiment

Source	Forward primer (5'-3')	Reverse primer (5'-3')
Emh11001	GATGTGTCGATGAGTTTTGGTCA	TAGCTACGTTGGTTTGGTGCTGAA
EMB01L13	TCAAAAGACTTGAAACCCGATGGT	GTTTATCAGGTTTTGATCACCGGACA
EMB01H20	TCTTGTTCCCAGTCTATATCGCTAATCA	ATCCGAATTTAGTCGGGCTTCAAT
emf21C11	TGGTTGGAGCCATGATTACTTGAA	ATGCTACCTATCAAACAGGCGGAA
emf21H22	CACAAGATGAAGACTAAGGAGTCC	CTTCTTCAACCTGTTCTTTAGCCCA
EEMS15	GGGACAAATCTGACCTTTGG	CTGGTGGCAAATTCTTCGAT
EEMS17	TGACATGTAGCTGGGCAGAG	TGGAGTGTGCATCCCAAATA
EEMS28	GACGATGACGACGCGATAA	TGGACTCACAACTCAGCCAG
EEMS48	CAATGCAAACAATTATCATTTCG	TCGATGTTGTTGTCGTCGTT
EEMS49	TGAAATTGATCAATACCTATAAATTTAG	GAAAGCCAGGATAGCATTCG
EM119	CCCCACCCCATTTGTGTTTATGTT	ACCCGAGAGCTATGGAGTGTTCTC
EM140	CCAAAACAATTTCCAGTGAGACAAGAGG	GACCAGAATGCCCCTCAAATTAAA
EM145	CAGTGCTACATAAATTGAGACAAGAGG	GGAGGTACAACGATTTTCATATCGGT
EM155	CAAAAGATAAAAAGCTGCCGGATG	CATGCGTGAGTTTTGGAGAGAGAG

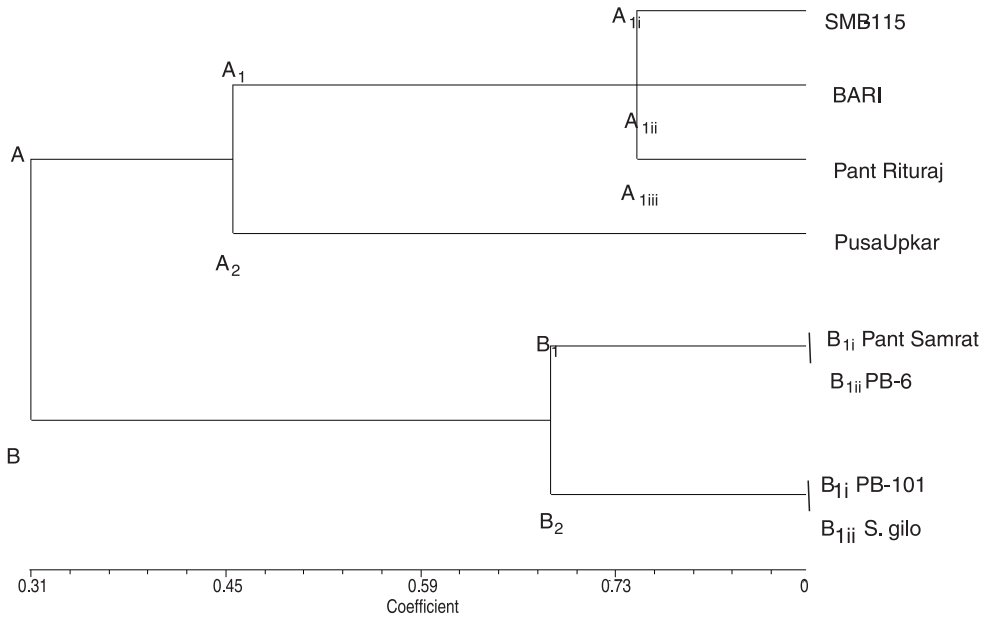

Fig 1 Classification of eight genotypes of eggplant constructed using UPGMA method and based on SSR. The scale at the bottom is Jaccard's coefficient of similarity.

similarity coefficient 0.45. A_1 contain three genotypes A_1i (SMB-115), A_1 ii (BARI), A_1 iii (Pant Rituraj) and A_2 contain only one genotype (Pusa Upkar) at Jaccard's similarity coefficient 0.88. Cluster B is divided in two sub clusters B_1 and B_2 at Jaccard's similarity coefficient of 0.70. B_1 again divided into sub cluster B_1i (Pant Samrat), B_1ii (PB-6) and B_2 also divide into two sub cluster B_2 i (PB-101) and B_2ii (*S. gilo*) at Jaccard's similarity coefficient 0.88. Genotype 1 (SMB-115) and 6 (PB-101) were most diverse among all the eight genotypes indicating that the cross between genotype SMB-115 and genotype PB-101has potential to give more heterotic hybrids due to their highly diverse nature.

SSR appear to be useful for taxonomic studies, diversity analysis and to establish relationship at levels ranging from populations to species and perhaps genera. SSRs studies in several different plant species have examined the relationship between candidate genes and quantitative variation.

Table 2 Jaccard's similarity coefficient matrix of SSR for eight brinjal genotypes

L1	L2	L3	L4	L5	L6	L7	L8
1.00							
0.75	1.00						
0.25	0.50	1.00					
0.75	0.75	0.25	1.00				
0.12	0.37	0.87	0.12	1.00			
0.62	0.37	0.37	0.37	0.50	1.00		
0.25	0.75	0.75	0.50	0.62	0.37	1.00	
0.12	0.12	0.62	0.37	0.75	0.50	0.87	1.00

L1, SMB-115; L2, BARI; L3, Pant Samrat, L4, Pant Rituraj; L5, PB-6; L6, Pusa Upkar; L7, PB-101; L8, *S. gilo.*

Diversity analysis helps to assess the genetic distance between the genotypes, hence after clustering suitable parents could be identified for *Solanum melongena* improvement programme through hybridization (Bered *et al.* 2005), due to the high resolution and reliability in the identification of cultivars. Despite the lack of direct correlation between the molecular diversity and the level of heterosis, diversity at DNA level forms a very useful guide not only for investigating the relationship among the Brinjal genotypes but also in the selection of parents for heterotic hybrid combinations. These results are in consonance with the findings of several workers like Staub and Serquen (1996), Powell *et al.* (1996), Jones *et al.* (1997), Nunome *et al.* (2003 a, 2003 b) and Varshney *et al.* (2005). Many more workers also reported the importance of SSR markers, in support of these finding and those report are; SSR is co-dominant in nature with a high information content (Danin-Poleg *et al.* 2000), SSR has facilitated the studies of genetic diversity (Plaschke *et al.* 1995), gene mapping by SSR markers (Roder *et al.* 1998, Pestsova *et al.* 2002), these report were similar to present findings. Similar results were also reported by Bora (2010) for the molecular diversity studies in 17 genotypes of *Solanum melongena* and on the line of *S. aethiopicum* based on microsatellite (SSR) markers. It can be concluded that high genetic diversity could be the result of differences among genotypes due to diversification in their pedigree. Our results were in conformity with the studies of Adeniji *et al.* (2012), Verma *et al.* (2012), Ansari and Singh (2014), Caguiatand Hautea (2014), Zhou *et al.* (2015), Mangal *et al.* (2016), Kaushal *et al.* (2017), Pungdilaand Khanna (2017), Boureima *et al.* (2018), Liu *et al.* (2018).

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