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Diversity Analysis in Brinjal (*Solanum melongena L.)* Genotypes Using Microsatellite Markers

Anita Pande *
College of Biotechnology, Birsa Agricultural University, Ranchi, India
Email: anitapande66@gmail.com

Bharti Kumari
College of Biotechnology, Birsa Agricultural University, Ranchi, India

Abdul Majid Ansari
Department of Horticulture, Birsa Agricultural University, Ranchi, India

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Abstract

Brinjal (*Solanum melongena* L.) is commonly called as eggplant or aubergine. It is one of the most important commercial solanaceous vegetable crops grown widely in Asian and African countries. It is popular among people of all social strata and is rightly called a vegetable of masses [1, 2]. It is of substantial economic importance in Asia, Africa, and the subtropics (India, Central America), and it is also grown in some warm temperate regions (Mediterranean area, Southern USA) [3, 4]. It is an old world species and native to Indo-Chinese centre of origin [5]. The genus *Solanum* is morphologically hypervariable and highly diverse in number of species [6] and ecogeographical distribution [7]. Despite the economic and nutritional importance of eggplant, breeding efforts in this crop have been limited compared to other solanaceous crops, such as tomato and potato [8, 9]. In the present study 30 solanaceae specific microsatellite markers were used of which 29 showed good amplification in the 4 checks used viz state check for round and oblong to long fruit, national check for round fruit and national check for oblong to long fruit. Of these 29, primer set emi04J02 and emk03O04 did show amplification in the other genotypes. Finally, 27 markers were used for analysis. Of these 23 showed monomorphic pattern (85.1%) whereas only 4 showed some polymorphism (14.8 %). The most informative marker was eme01D03 which showed presence of 3 alleles. The low degree of polymorphism indicated a high degree of homogeneity in the genotypes which could be due to a narrow genetic base.

Keywords: Brinjal; Solanaceae; Microsatellite markers; Polymorphism.

1. Introduction

Brinjal is largely made up of water and is a high-fibre, low-calorie food rich in vitamins and minerals especially iron, compared to other commonly consumed vegetables. It is considered as a poor man's vegetable and nutritionally comparable to tomato [10]. It contains proteins, carbohydrates, fibers and vitamins like thiamine, niacin, pantothenic acid and folacin as well as minerals like calcium, iron, potash, zinc, copper and manganese. It is one of the top 10 vegetable in terms of antioxidant capacity because of the presence of anthocyanin, which is an important antioxidant with a variety of physiological functions such as anti-mutagenesis, anti-inflammatory and anticancer properties. Due to its outstanding health benefits, it is considered as a model vegetable crop to the researchers [11, 12]. Despite the economic and nutritional importance of eggplant, breeding efforts in this crop are limited.

The study of morphological characters is helpful in assessing similarities and dissimilarities among genotypes selection and for breeding purposes [13]. DNA based markers are superior to morphological markers as they are highly reproducible, can be developed at any stage of plant growth and only a small amount of plant tissue is needed. Traits such as insect and disease resistance can be selected by a breeder in absence of the pests with a marker are

linked to it. This allows reliable tracking of beneficial traits during selection. Multiple beneficial genes can be detected in a short time at the seedling stage itself, thus saving time, energy and money. Molecular characterization of germplasms also helps in assessment of genetic variability which can aid in the crop improvement program as the genetic diversity or relatedness can be assessed [14]. Development of SSR primers is a tedious and expensive process; therefore heterologous markers may be employed in crops for which they are not available. Nunome, *et al.* [15] were the first to use SSR markers for studying molecular diversity among the genotypes of brinjal. They concluded that SSRs were useful for genetic analysis of brinjal and could also be used for marker assisted breeding (MAB) programs. With this background, 48 genotypes which included 4 checks were used in the present study for assessment of diversity which would be helpful in formulation of breeding programmes.

2. Materials and Methods

Forty-eight genotypes of brinjal were acquired from Department of Horticulture, Birsa Agricultural University (BAU), Ranchi. These included 4 checks (Table 1).

Table-1. Important traits of 48 Brinjal genotypes

S. No.	Entry	Stem colour	Leaf colour	Mid rib colour	fruit colour	prickles on midrib	prickles on epicalyx	prickles on leaves	prickles on stem	prickles on petiole	Colour of epicalyx	types of epicalyx	Fruit Shape
1	CB-1 (Birsa Chianki Baigan-1)	PG	PG	P	P	X	x	X	x	X	PG	Fleshy	Egg shaped
2	CB-2	PG	PG	P	P	X	x	X	x	X	PG	Fleshy	Egg shaped
3	CB-3	PG	G	P	P	X	x	X	x	X	PG	Fleshy	Egg shaped
4	CB-4	G	G	LG	LG	X	x	X	x	X	G	Fleshy	Oblong
5	CB-5	PG	DG	P	P	X	x	X	x	X	PG	Fleshy	Oblong
6	CB-6	PG	DG	PG	PGD	X	x	X	x	X	PG	Fleshy	Oblong
7	CB-7	PG	DG	PG	P	X	x	X	x	X	PG	Fleshy	Oblong
8	CB-8	PG	DG	PG	P	X	x	X	x	X	G	Fleshy	Oblong
9	CB-9	G	G	LG	P	X	x	X	x	X	PG	Leathery	Oblong
10	CB-10	PG	G	PG	LP	X	x	X	x	X	PG	Fleshy	Long
11	CB-11	PG	G	PG	LP	X	x	X	x	X	PG	Fleshy	Long
12	CB-12	PG	G	LP	LP	X	x	X	x	X	PG	Fleshy	Long
13	CB-14	G	LG	LG	LG	X	x	X	x	X	G	Fleshy	Long
14	CB-15	G	LG	LG	LG	X	x	X	x	X	G	Fleshy	Oblong
15	CB-16	G	LG	LG	LG	√	√	√	√	√	G	Leathery	Long
16	CB-17	G	LG	LG	LG	X	X	X	x	X	G	Leathery	Long
17	CB-18	PG	DG	P	P	X	X	X	x	X	PG	Leathery	Oblong
18	CB-19	G	G	LP	G	X	X	X	x	X	G	Leathery	Egg shaped
19	CB-20	G	G	LP	LP	X	X	X	x	X	PG	Leathery	Oblong
20	CB-21	G	G	LG	LP	X	X	X	x	X	PG	Leathery	Oblong
21	CB-22	G	G	LG	LG	X	X	X	x	X	LG	Papery	Egg shaped
22	CB-24	LP	G	LP	LG	X	X	X	x	X	PG	Papery	Egg shaped
23	CB-25	PG	G	G	LGP	X	X	X	x	X	G	Papery	Egg shaped
24	CB-26	PG	PG	P	P	X	X	X	x	X	PG	Fleshy	Egg shaped
25	CB-27 (Birsa Chianki Baigan-2)	PG	DP	P	P	X	X	X	x	X	PG	Fleshy	Egg shaped
26	CB-28	G	G	LG	LG	X	X	X	x	X	G	Fleshy	Egg Shaped
27	CB-31	PG	DG	P	LG	X	√	X	√	X	G	Fleshy	Oblong
28	CB-34	G	LG	LG	LG	X	X	X	x	X	G	Fleshy	Long
29	CB-36	PG	DG	P	P	X	X	X	√	X	PG	Fleshy	Long
30	CB-38	PG	PG	P	P	X	X	X	√	X	PG	Papery	Oblong
31	CB-41 (Birsa Chianki Baigan-3)	PG	G	LP	LG	X	√	X	√	X	G	Papery	Oblong
32	CB-47	PG	DG	LP	LG	X	√	X	√	X	PG	Fleshy	Egg shaped
33	CB-55	PG	DG	LP	LPG	√	√	√	√	√	PG	Papery	Round
34	CB-58	PG	DG	LP	LP	X	√	X	√	X	PG	Papery	Egg shaped
35	CB-59	LPG	DG	G	G	X	√	X	x	X	PG	Fleshy	Egg shaped
36	CB-62	PG	DG	P	PG	X	X	X	x	X	PG	Fleshy	Oblong
37	CB-63	PG	DG	P	PG	X	√	X	x	X	PG	Fleshy	Long
38	CB-64	PG	DG	P	PG	X	X	X	x	X	PG	Fleshy	Long
39	CS-1	PG	G	LPG	LG	X	X	x	x	X	G	Papery	Egg

													shaped
40	CS-3	PG	DG	LP	PW	X	X	x	x	X	PG	Papery	Round
41	CS-7	PG	DG	P	LP	X	X	x	x	X	PG	Papery	Long
42	CS-12	PG	DG	P	P	X	X	x	x	X	PG	Fleshy	Oblong
43	LC-3 (Selection from local collection)	PG	DG	LP	P	X	√	x	√	X	PG	Fleshy	Oblong
44	Lal Gulab Selection	G	G	LG	LP	X	X	x	x	X	PG	Fleshy	Long
45	Swarna Shyamli (State Check for round)	PG	DG	LP	G	√	√	√	√	√	G	Fleshy	Round
46	Swarna Pratibha (State Check for oblong to long)	PG	DG	P	P	X	X	x	x	X	PG	Fleshy	Oblong to long
47	PusaUpkar (National Check for round)	PG	PG	P	P	X	√	x	x	X	PG	Papery	Round
48	Pant Samrat (National Check for oblong to ,long)	PG	G	P		X	X	x	x	X	PG	Papery	Oblong to Long

KEY: CB = Chianki Brinjal, CB = Chianki Selection, LC= Local Collection, PG = Purple Green, LG = Light Green, LP = Light Purple, PGD = Purple Green Dark, DG = Dark Green, G = Green, LPG = Light Purple Green, LGP = Light Green Purple

- **Breeding method used for development of genotypes:**
  - 1-38: -Pedigree selection method
  - 39-44: - Pure line selection method
- **Origin of genotypes:**
  - 1-44: - Zonal Research Stations (ZRS), Chianki (BAU)
  - 45-46:-Indian Council of Agricultural Research- Research Complex for Eastern Region Research Centre (ICAR-RCERRC), Plandu
  - 47:- Indian Agricultural Research Institute (IARI), Pusa, New Delhi
  - 48:- Govind Ballabh Pant University of Agriculture and Technology (GBPUAT), Pantnagar

3. Isolation and Purification of DNA

3.1. Collection of Leaf Samples

2 gm of young and healthy green leaves of brinjal genotypes were collected. All samples were encased in aluminum foil, labelled and stored on ice packs for transportation to the lab. The packets were then snap frozen in liquid nitrogen and stored at -80^{o}C till further use.

3.2. DNA Extraction

The extraction of plant genomic DNA from the collected samples was done using Cetyl Trimethyl Ammonium Bromide (CTAB) method [16].

3.3. SSR Primers

For analysis of the DNA, 30 different solanaceae microsatellite primer sets were obtained based on the information provided by National Bureau of Plant Genetic Resources (NBPGR), New Delhi (Table 2).

Table-2. Details of polymorphic SSR markers

S. N	Name Source	Repeat type and length	Chromosme No.	Forward primer sequence	Reverse primer sequence
01	emi04J02	$(TA)_8T(AC)_9$	1	ACAGAAGCCTTGGCTTATATGATGA	GTTTCCCGAGAGGTTGCTACTGTAGACG
02	emg11M21	$(AG)_{23}AA(AG)_3$	1	ATAGCCTACTGCCTTCAAGACCAT	GTTTCCTACGTCCAGTCCCCTTAGGT
03	emh05B02	$(AG)_{43}$	3	ATACCAAAGACACGTTGGGATCAT	GTTTCTAGGAGAGCATCTCCCTCCCT
04	emf01O04	$(TA)_{10}(TG)_{20}$	4	ATCCGTTGATACTAGCCGTTGCCT	GTTTCACCCGGTATGAGTGTATCCC
05	emk04N11	$(AC)_{15}GC(AC)_6A(TA)_9$	4	ATCTCCCCCTCAACTTTGAACAAT	GTTTGTGTGATATAGCCCAACAATTCAC
06	emk03O04	$(AC)_{23}A(TA)_9CA(T$	4	ATGATTTGGGCAGCC	GTTTGGAACCAACTAAACT

		$A)_{12}$		ACTTTTGTA	TAGGGCA
07	emf21C11	$(TC)_{24}$	4	AGGTTGGAGCCATGA TTACTTGAA	GTTTGCTACCTATCAAACA GGCGGAA
08	emf21I02	$(TC)_{20}$	7	AGTGCATTTCTCAAAT CAAAAGGG	GTTTCAATTTCACAGGCTC CTGCATTA
09	emf21H22	$(AG)_{12}AA(AG)_{18}$	7	ATCAAGATGAACAAG ACTAAGGAGTGC	GTTTCTTCAACCTGTCTTTA GCCCA
10	emb01L13	$(AG)_6$	8	TCAAAAGACTTGAAA CCCGATGGT	ATCAGGTTTTTGATCACCG GACA
11	emd05F08	$(TA)_5(CA)_{10}$	10	ACAGGCAACCAAGTT ACCAACCCTTT	GTTTAAAATGTCCGGTTAT GGATTCGC
12	eme10G12	$(TC)_{15}TT(TC)_4$	8	ATCCACTTAGACCATT GCCCTCTTC	GTTTCCGACTTTTGCCAAC TACGTAAGC
13	eme11C02	$(AG)_3AA(AG)_3T(GA)_{13}$	8	ACGAGAGAGAGAGAG AGGGCATGGAA	GTTTGATGTTAGAGGCGAG CATGTGT
14	eme11f04	$(TC)_{16}$	8	ACCCCCAAATCAAAT CATTTACCC	GTTTGGCATGGTTAGGGTT TTTAGCGTT
15	emd03E08	$(TA)_6(TG)_9$	7	ACGTACGCATGCTGTG TTTGTTAGGC	GTTTGGACACCCGGAGACA ATCTACTTT
16	eme08D09	(TC)5C(CT)20T(TC)12(AC)5	7	ATGGATTAGCATGTG GAGGACTGAA	GTTTCATGGTAGGTGGAGA CAGAACCA
17	emf01K16	(TC)9(TG)13...(TG)4	5	ATTTGGACAAGAACA AGGATGGCT	GTTTCACTCACAATTCGAG ACACTCGGT
18	eme05G05	(AG)3...(GA)6	5	ACAAGAAAGAGGAGC TGGGGAAATTG	GTTTCCTTCTTGGGAAGAC AACTTATCA
19	emd06A03	(CA)14(TA)4...(TA)3	4	ACCCAAACCCTGCAT ACAAAAGAA	GTTTAGGTTTGTGGAATCC TGTGCGTAT
20	emd02A05	(AC)10(AT)4	4	ACCATTGTACCCCTGA ACGGATATACA	GTTTCCGATGTACAGTTTG TTGACCCAC
21	eme10H05	(AG)16	3	ACAAGACGAAAGTGT GCAGACCAG	GTTTGAAAGTGAAGAGTCC GTGCAGT
22	eme03H10	(AG)17	3	ATGGAATTGTTCCCTG AAGTCCTGT	GTTTCCCAAGCCTGCAATC TTTAACATC
23	emd05B11	(TC)20(TG)13...(TC)4C(CT)3	1	ATTGCTTCAATTAAGG CTGAGAGGG	GTTTATTTTTCGCTTGAGA GTGGTGGC
24	eme01D03	(AG)15	1	ACAAGAATCGGTCCT CTTTGCATTGT	GTTTGCTTTTCACCTCTCCG CTATCTC
25	emb01H20	(TC)21	9	TCTTGTTCCCAGTCTA TCGCTAATCA	ATCCGAATTTAGTCGGGCT TCAAT
26	emb01O01	(AG)18	11	TTAACATCGCCGTTGG CTTCTTAG	GTTTCGATAACCAAAAGGG GTTTCAACA
27	eme11G05	(AG)10	5	ACTCGTCTCATGGAGC GATATTGTG	GTTTCCTTGCTTTTTGTGAT GCAGATTG
28	emd12B05	(AC)33(AT)9	8	ACGGAGTAGGCTCGG AGCGTGATATT	GTTTGAAAGGGCAAAAAG TCCAAACAAC
29	emj04D04	(TA)3...(TA)15	1	ATTGACCTAGCCCTCT TAGGCGAC	GTTTATGTTGGAAGGTTTA ACCGCAGC
30	eme01B01	(AG)21	1	ATGTGCATCTCTTGTT GGTAGGAGC	GTTTCTCGGATTGATCCCA AAGGGTACT

The markers are mapped to different locations on the brinjal genome [15].

3.4. Gradient PCR for Determination of Best Annealing Temperature

A gradient PCR was set up for 30 primers with genotypes 45, 46, 47, 48 to estimate the best annealing temperature to be used. Three different annealing temperature 50°C, 55°C and 60°C were used to check the amplification with further refining , if needed. Amplification was executed in a programmable thermal cycler (Eppendorf). The PCR was set up in a 10 µl reaction mix with 50ng template DNA, 1 µl of Thermo Fisher green buffer, 200 µM of each dNTP, 5pmol each of forward and reverse primer and 0.5 unit of Thermo Fisher Dream Taq. The remaining volume was made up with sterile distilled water. After determination of the best annealing temperature for each SSR all 48 genotypes were amplified with Initial denaturation at 94°C for 4 minutes, 30 cycles of denaturation at 94°C for 30s, annealing at 50 to 60°C for 30s and extension at 72°C for 30s. A final extension was given for 2 minutes at 72°C.

3. 5. Agarose Gel Electrophoresis of SSR Products

The amplicons obtained after PCR were run on 2% agarose gel. The gel was prepared by dissolving 6g agarose in 300ml TAE buffer. After dissolution of the agarose, 15 µl of ethidium bromide (10mg/ml) was added and gently mixed to avoid air bubbles. The electrophoresis was carried out at constant voltage (5v/cm) and the image was captured using an Alphadigidoc system.

4. Results

The table below shows the best annealing temperature obtained for each SSR:
No amplification was found with SSR # 14 viz. eme11f04.

Primer No.	Annealing temperature	Primer No.	Annealing temperature
1	60˚C	16	55˚C
2	60˚C	17	57˚C
3	60˚C	18	57˚C
4	55˚C	19	60˚C
5	55˚C	20	55˚C
6	47˚C	21	57˚C
7	57˚C	22	57˚C
8	60˚C	23	57˚C
9	60˚C	24	57˚C
10	60˚C	25	57˚C
11	60˚C	26	57˚C
12	60˚C	27	57˚C
13	57˚C	28	57˚C
14	_	29	57˚C
15	57˚C	30	57˚C

The analysis of 48 genotypes with the 29 primers selected for this study revealed that 23 primers showed a monomorphic pattern and only 4 primers were distinctly polymorphic. Primer 1 and 6 did not show good amplification. Plate 1 depicts the monomorphic pattern obtained with SSR 11 viz. emd05F08.

Primer set 16 (eme08D09), 18 (eme05G05), 19 (emd06A03), and 24 (eme01D03) showed a polymorphic pattern. The first three showed presence of mainly 2 alleles each whereas eme01D03 showed presence of 3 alleles Plate 2)

Primer set 16 showed the presence of one major allele across all genotypes. A small minor allele was found in 29 genotypes. The difference between the two alleles according to the repeat size (Table 2) should be in multiple of 86 bp. The major and minor allele compared with the 50 bp ladder indicated that there is a difference of a single repeat between the two. A single genotype, CB-15, showed the presence of multiple alleles. SSRs are multiallelic and multilocus markers. Thus, Primer set 16 could be used to distinguish genotype CB-15 from others after further refining and serve as a fingerprint.

Three major alleles were observed with Primer set 24 i.e., eme01D03 (Plate 2). The alleles were labeled as a, b and c. Allele b was found in all genotypes except 21 and 22 which were CB-22 and CB-24. These two genotypes, in fact, showed no amplification with any SSR.

Further analysis was attempted based on the presence of these 3 alleles. Those genotypes which showed the presence of allele ‘a’ and their phenotypic characteristics are shown in Table 3 and those of allele c are shown in Table 4

Table-3. Phenotypic traits of genotypes showing Allele a:

Traits	Genotypes								
	CB-11	**CB-12**	**CB-17**	**CB-19**	**CB-20**	**CB-21**	**CB-64**	**CS-1**	**Pant Samrat**
Stem colour	PG	PG	G	G	G	G	PG	PG	PG
Leaf colour	G	G	LG	G	G	G	DG	G	G
Midrib colour	PG	LP	LG	LP	LP	LG	P	LPG	P
Fruit colour	LP	LP	LG	G	LP	LP	PG	LG	DP
Colour of epicalyx	PG	PG	G	G	PG	PG	PG	G	PG
Type of epicalyx	Fleshy	Fleshy	Leathery	Leathery	Leathery	Leathery	Fleshy	Papery	Papery
Fruit shape	Long	Long	Long	Egg shaped	Oblong	Oblong	Long	Egg shaped	Oblong to long

Table-4. Phenotypic traits of genotypes showing Allele c:

Traits	Genotypes								
	CB-3	**CB-4**	**CB-5**	**CB-8**	**CB-12**	**CB-17**	**CB-36**	**CS-3**	**Pant Samrat**
Stem colour	PG	G	PG	PG	PG	G	PG	PG	PG
Leaf colour	G	G	DG	DG	G	LG	DG	DG	G
Midrib colour	P	LG	P	PG	LP	LG	P	LP	P
Fruit colour	P	LG	P	P	LP	LG	P	PW	DP

Colour of epicalyx	PG	G	PG	G	PG	G	PG	PG	PG
Type of epicalyx	Fleshy	Fleshy	Fleshy	Fleshy	Fleshy	Leathery	Fleshy	Papery	Papery
Fruit shape	Egg shaped	Oblong	Oblong	Oblong	Long	Long	Long	Round	Oblong to long

Key: G = Green, PG = Purple Green, LG = Light Green, LP = Light Purple,
DG = Dark Green, LPG = Light Purple Green, P = Purple, DP= Dark Purple

None of the trait was common in all the genotypes. The genotypes which showed the presence of both allele 'a' and 'c' are shown in Table 5.

Table-5. Phenotypic traits of genotypes showing Allele 'a' and 'c':

Traits	**Genotypes**		
	CB-12	**Pant Samrat**	**CB-17**
Stem colour	PG	PG	G
Leaf colour	G	G	LG
Midrib colour	LP	P	LG
Fruit colour	LP	DP	LG
Colour of epicalyx	PG	PG	G
Types of epicalyx	Fleshy	Papery	Leathery
Fruit shape	Long	Oblong to long	Oblong

Key: G = Green, PG = Purple Green, LG = Light Green, LP = Light Purple, P = Purple, DP= Dark Purple

Pant Samrat is the national check for oblong to long fruit shape. The fruit shape of CB-12 is long and CB-17 is oblong. CB-12 is closer to Pant Samrat on the basis of allele 'a' and 'c' as there is some similarity between them. Stem colour is purple green, leaf colour is green, colour of epicalyx is purple green but this is not sufficient to find any kind of relatedness or closeness.

5. Discussion

Of the 27 primers that could be analysed in the present study 23 showed a monomorphic pattern. Therefore, the percentage of polymorphic markers is 14.8% which is very low. In all, it was observed that a great deal of homogeneity is present in all the genotypes. The information of pedigree of the genotypes is not available (withheld). It appears that the selection has been made from a narrow base. However, other studies have also found that the degree of polymorphism to be low in brinjal. Khorsheduzzaman, *et al.* [14], studied five brinjal genotypes using 11 RAPD markers. Of the 22 bands obtained from 3 markers, 15 were polymorphic. Based on the value of Jaccard's coefficient, genetic similarities between SSR profiles were assessed. The similarity value ranged from 0.83 to 1.00, indicating a limited range of genetic variety at the molecular level. Demir, *et al.* [17], conducted an experiment using SSR and RAPD markers on brinjal collected from different geographical regions of Turkey and they found that most of the primers gave less than 50% polymorphism. Alessandro, *et al.* [18], studied genetic diversity among 70 scarlet eggplant (S. aethiopicum L.) entries from different geographical origins. They used amplified fragment length polymorphism (AFLP) and SSR markers. A dendrogram was created using genetic similarity matrices from AFLP and SSR data. The cluster that had multiple South American entries showed very low rates of genetic variation. The cluster of entries from Africa, of which some were common to the accessions collected in Italy, showed a higher level of diversity. Adeniji, *et al.* [19], investigated 7 Solanum species consisting of for 39 Solanum accessions, a landrace and a tomato variety (LBR 48) using SSR. A total of 417 alleles were amplified, ranging from 5 to 38 alleles per SSR. The dendrogram produced based Jaccard's coefficient of similarity and UPGMA clustering showed that entries from various regions of the world did not cluster together, and there was no correlation between the geographic origin and the SSR marker pattern.

SSR are multi-allelic in nature. The study employed SSRs from *Solanaceae* family mainly for brinjal and tomato. They were repeat sequence which ranged from 12 to 86 repeats (Table 2). Most repeats were compound repeats (SSR containing stretches of two or more different repeats) and compound repeats show lesser degree of variations (Kumar *et al.*, 2015). The SSRs were well distributed on the brinjal genome (Nunome *et al.*, 2003). Despite the good distribution on all the chromosomes, very low polymorphism was obtained indicating that the genetic base is very narrow. Other markers such as single nucleotide polymorphisms (SNPs) may prove more useful in uncovering polymorphisms in this crop.

A recent study has shown the genome size of the eggplant inbred line HQ-1315 to be approximately 1205.25 Mb, with a heterozygosity rate of 0.15%, as assessed by k-mer analysis based on 93.33 Gb Illumina HiSeq data [20]. This high-quality reference genome has a total size of ~1.17 Gb and 12 chromosomes consisting of 36,582 protein-coding genes. Repetitive sequences comprise 70.09% (811.14 Mb) of the eggplant genome. Comparative analysis of different eggplant genomes identified three types of variations, including SNPs, insertions/deletions (indels) and structural variants (SVs). Asymmetric SV accumulation was found in potential regulatory regions of protein-coding genes among the different eggplant genomes. Information generated in this study can be used for *in silico* detection of polymorphism.

6. Conclusion

The present study indicated that though the 48 genotypes used differed vastly at the morphological level, the same did not reflect in the degree of polymorphism observed with SSR markers. The usage of simple repeats/increasing the number of markers/using different markers uncovered through *in silico* studies may yield

higher polymorphism which could then be exploited for designing marker assisted breeding programmes. Wei, *et al.* [20], have predicted a key candidate gene for eggplant fruit length regulation viz. *Smechr0301963*, which belongs to the *SUN* gene family. Our study had genotypes of different fruit length and shape. Unravelling of markers linked to such traits is important for further research on brinjal. More intensive study to link traits with molecular markers is thus warranted.

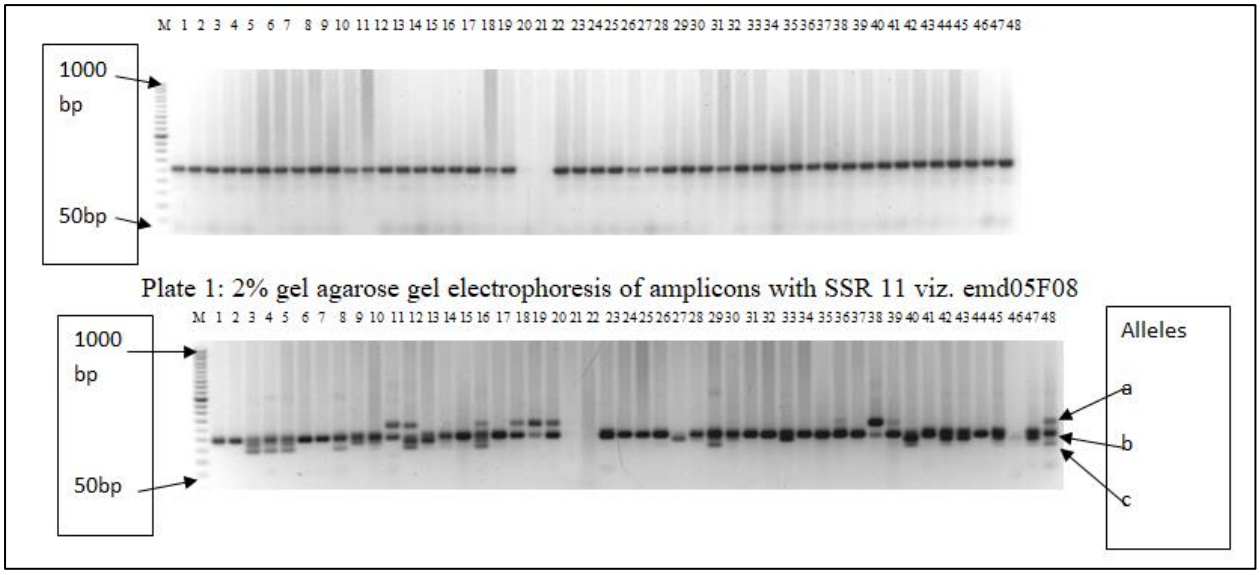

Plate-1. 2% gel agarose gel electrophoresis of amplicons with SSR 11 viz. emd05F08

M 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 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48

Plate 2: 2% gel agarose gel electrophoresis of amplicons with SSR 24 viz. eme01D03

M: 50bp Marker, Lane 1: CB-1, Lane 2: CB-2, Lane 3: CB-3, Lane 4: CB-4, Lane 5: CB-5, Lane 6: CB-6, Lane 7: CB-7, Lane 8: CB-8, Lane 9: CB-9, Lane 10: CB-10, Lane 11: CB-11, Lane 12: CB-12, Lane 13: CB-14, Lane 14: CB-15,Lane 15: CB-16, Lane 16: CB-17,Lane 18: CB-19, Lane 19: CB-20, Lane 20: CB-21, Lane 21: CB-22, Lane 22: CB-24, Lane 23: CB-25, Lane 24: CB-26, Lane 25: CB-27, Lane 26: CB-28, Lane 27: CB-31,Lane 28: CB-34,Lane 29: CB-36,Lane 24: CB-26,Lane 25: CB-27, Lane 26: CB-28, Lane 27: CB-31, Lane 28: CB-34, Lane 29: CB-36,Lane 30: CB-38, Lane 31: CB-41, Lane 32: CB-47,Lane 33: CB-55, Lane 34: CB-58,Lane 35: CB-59,Lane 36: CB-62,Lane 37: CB-63, Lane 38: CB-64, Lane 39: CS-1, Lane 40: CS-3, Lane 41: CS-7, Lane 42: CS-12, Lane 43: LC- 3, Lane 44: Lal Gulab Selection, Lane 45: Swarna Shyamli, Lane 46: Swarna Pratibha, Lane 47: Pusa Upkar, Lane 48: Pant Samrat.

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