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SSR BASED MOLECULAR STUDY FOR RATOONING EFFECT IN *AETHIOPICUM* AND *MELONGENA* SPECIES OF THE GENUS *SOLANUM*

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ABSTRACT Thirteen genotypes of *melongena* and one genotype of *aethiopicum* of the genus *Solanum* were undertaken to study the ratooning effect and its molecular relationship using fourteen pairs of SSR primers. Analyzed data revealed that the mean performance of different genotypes of the genus *Solanum* for fruit characters *viz*., fruit length, fruit diameter; average fruit weight, number of fruits/plant and weight of healthy fruits/plant were decreased in the ratoon crop as compared to main season crop and yield/plant and yield/hectare were also reduced in the range of 39.52 % (LC-7) to 97.40% (KS-331). Amplification of genomic DNA of fourteen genotypes using fourteen pair of SSR primer exhibited a clear cut differentiation among the genotypes through electrophoresis gel with the range of similarity 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 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 which revealed that morphological characters *viz.*, shape, size and peel colour of fruits and plant type showed a positive relationship with the DNA based molecular analysis through SSR markers. SSR primer EM140 differentiated the brinjal genotypes in response to ratooning effect by producing double band in the genotypes BARI, PB-66, Pant Rituraj, PB-71, Pant Samrat, NDB-1, SMB-115 and KS-331 which were performing better under ratooning condition except, NDB-1.

KEYWORDS: *Solanum, aethiopicum, melongena*, brinjal, ratooning, SSR markers.

INTRODUCTION

Brinjal (*Solanum melongena* L.) is known as eggplant in United States and *aubergine* in France and England. It is one of the important vegetable crops 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 centre of origin and diversity of eggplant (Vavilov, 1951 and Isshiki *et al*., 1994a, b, c). 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 use as well as breeding purposes. In Africa, many *Solanum* species are used for medicinal purposes (Bukenya and Carasco, 1999) such as *S. aethiopicum* group *gilo* and *S. anguri.* The 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 (Danin-Poleg *et al*., 2000). It is typically multi-allelic marker (Matsuoka *et al*., 2002) with heterozygosity values much higher than those of RFLPs (Mc Couch, 1997). SSR has facilitated the studies of genetic diversity (Plaschke *et al*., 1995), gene mapping (Roder *et al*., 1998; Pestsova *et al*., 2002) and testing of authenticity of genetic stocks (Pestsova *et al*., 2002). Co-dominant inheritance, abundance, wide genome coverage and multi-allelic nature, simple sequence repeats (SSRs) or microsatellites have now become the marker of choice (Frary *et al*. 2005; Sarıkamış *et al*. 2010). A number of SSR markers have been identified in *Solanaceae* (Yi *et al*. 2006; Bindler *et al*. 2007), but the numbers are less in eggplant. The development of SSR markers derived from SSR-enriched genomic library of eggplant has been reported by Nunome *et al*. (2003, 2009). SSR markers have been used in determination of genetic diversity in eggplant (Nunome *et al.* 2003a, 2003b; Stagel *et al*. 2008; Nunome *et al*. 2009). Cultural practices for production of early summer crops with lesser inputs are a need of farmers. In several crop plants, it is common to cultivate the ratoon crops as a subsequent crops *in situ viz.*, in banana (Irizarry *et al*., 1992), rice (Coale and Jones, 1994), sorghum (Mc Cromick *et al*., 1995), sugarcane (Yadava *et al*., 1994), pigeonpea (Chauhan *et al*., 1996) and lima bean (Beverly and Byns, 1992). Among the family *Solanaceae*

ratooning has been practiced on eggplant (Dhankhar *et al*., 1980) but, very few scientific studies has made on eggplant under this context. Therefore, present study was carried out to identify the most suitable genotypes for ratooning effect and SSR markers were undertaken to study molecular relationship of ratooning effect among the genotypes of *aethiopicum* and *melongena* species of the genus *Solanum*.

MATERIALS AND METHODS

The field trial was conducted at Vegetable Research Center (VRC), G. B. Pant University of Agric. & Tech. (GBPUA&T), Pantnagar (India) during *autumn-winter* (2010-11) as a main crop and in *spring-summer season* (2011) as a ratoon crop whereas, 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. VRC is geographically situated at an altitude of 243.84 meters above mean sea level and at 29^0 N latitude and 79.3^0 E longitudes. This falls in the humid sub-tropical zone and situated in the *Tarai* belt in the foothills of *Shivalik* range of the great Himalayas. Maximum temperature was ranging from 32^0 C to 43^0 C in summer and minimum temperature ranging from 0^0 C to 9^0 C in winter. Frost can be expected from last week of December to first week of February. Soil of the field was clay-loam in nature with rich in organic matter.

Ratooning:

The experimental material consisted of 14 genotypes of eggplant *viz*., BARI, PB-66, Pant Rituraj (PR), WB-1 (land race), PB-6, PB-71, Pant Samrat (PS), NDB-1, PB-70, PB-4, SMB-115, KS-331 and LC-7 of *S. melongena* and one genotype of *S. aethiopicum* (table 1*)*. Experiment was laid out in Randomized Block Design (RBD) with three replications. For main season crop, one month old seedlings were transplanted in the first week of July, 2010 at the spacing of 75 cm x 60 cm in three rows of 6 meters length constituted 10 plants each row, altogether 30 plants to each treatment. Recommended package of practices were followed for raising the normal seedlings and crops for both the conditions. Ratooning was done at the height of 30 cm to all the plants *in situ* in the first week of March, 2011. All plant debris, dried leaves and twigs were removed from the fields. Certain plant protection measures *viz.*, application of copper oxychloride @ 2 g/liter of water and Imidacloprid @ 4.2 ml/10 liter of water were sprayed just after cutting of branches. After 10 days of pruning, Carbendazim @ 1 g/liter of water and Triazophos @ 2 ml/liter of water were sprayed in the ratoon crops. Data's were recorded for Yield and its attributing characters during *autumn-winter* (2010-11) as a main crop and in *spring-summer season* (2011) as a ratoon crop to study ratooning effect in eggplant.

PCR based molecular analysis:

SSR technique was used to reveal molecular analysis among 14 genotypes of eggplant. Material used in the experiment was kit-extracted DNAs of 2-3 weeks old seedlings of eggplant collected from nursery especially sown for DNA extraction from leaves. High molecular weight genomic DNA was extracted for molecular biology work with the help of 'plant DNA isolation kit (CTAB method)' a product of *HiMedia* Lab. Pvt. Ltd. especially designed for plant genomic DNA isolation. Spectro-photometric analysis and *Agarose* gel-electrophoresis was used to check the quality and quantity of the genomic DNA. Elution buffer (ET) was used to dilute the samples and to calibrate the spectrophotometer.

PCR Amplification:

PCR amplification was performed in a volume of 25 µl reaction set up as table 2 and a set of crop specific 14 SSR primers pair was used for PCR amplification (Table 5).

Preparation for PCR Amplification:

A master mix without DNA template was prepared for different tubes to reduce pipetting error. The master mix was then redistributed in each PCR tube (23 µl each) and finally 2.0 µl of different DNA template was added in each tube. The content was gently mixed by centrifugation for one minute. The PCR amplification was achieved in an Eppendorf DNA thermo-cycler programmed (table 3) as follows:

***Agarose* Gel Electrophoresis:**

PCR amplified DNA fragments were resolved by submerged horizontal electrophoresis. *Agarose* gel (2-3%) was prepared by dissolving appropriate amount of *Agarose* in 1 X TAE buffer. For each well, amplified product and DNA loading dye were mixed in 3:1 ratio and loaded with a micropipette. Electrophoresis was done at 80 V for 3-4 hours in 1 X TAE buffer. Ethidium bromide solution (0.5 mg/ml) was added directly to the *Agarose* solution prior to casting at about $55\text{-}60^0$C. After completion of electrophoresis, image of the gel was viewed and saved in a gel documentation system (Alpha Imager EC).

Cluster analysis of banding pattern and scoring the gel:

The PCR amplification products were visualized in gel documentation system and photographs were saved for the analysis of genetic diversity. The amplification products were scored separately for each primer on the basis of presence or absence of band corresponding to each accession *i.e.*, use of binary code 1 and 0 for the presence or absence of band respectively. Molecular size (bp) of amplified DNA fragments were determined by the DNA ladder marker which was used in one well of *Agarose* gel. On the basis of absence and presence of SSR band and statistical data, similarity coefficient matrix among the 14 brinjal accessions was calculated by following Jaccard similarity index (1908). Dendrogram of accessions was constructed on the basis of presence or absence of SSRs band with the help of SAHN clustering analysis. All the numerical taxonomic analysis with respect to SSR (DNA fragment analysis) was performed using the NTSYS-pc, 2.1software (Rohlf, 2000). The field data were compared with the banding pattern of different SSR markers. Presence or absence of SSR band in the gel electrophoresis was compared to study the genotypes of *Solanum* species using SSR marker for its response to ratooning effect.

RESULTS AND DISCUSSION

Ratooning effect:

The mean performance of fruit characters *viz.*, fruit length, fruit diameter, average fruit weight, number of fruits/plant and weight of healthy fruits/plant were decreased in the ratoon crops as compared to main crops except some genotypes increased for certain trait viz., WB-1 for fruit length by 134.07%, KS-331 for fruit diameter by 131.56%, average fruit weight by 167.88% and for weight of infested fruits/plant by 253.99%, Pant Rituraj and PB-70 for number of fruits/plant by 104.35% and 104% 253.99%, respectively. Whereas, the weight of infested fruits/plant varied in the ratooning condition, ranging from 26.39% (*S. aethiopicum*) to 253.99% (KS-331) and mean value was 102.41 % (table 4). Yield/plant and yield/hectare were also reduced in the range of 39.52% (LC-7) to 97.40% (KS-331) and mean value was 66.26% (table 4). In the main crops, significantly highest yield was recorded in the genotype PB-70 (534.81 q/ha) followed by LC-7 (513.77 q/ha) which was statistically at par whereas, in the ratoon crops, it was significantly superior in the genotype KS-331 (434.44 q/ha), which was 97.59 % yield of the main crop (table 4). The mean values in ratoon condition were altogether reduced as compared to the main crops (Dhankhar *et al*., 1980), except weight of infested fruits per plant which was increased by 102.41% that is not desirable (table 4). These results are in agreement with the findings of Sigh *et al*. (2011).

Table 1: List of genotypes of *Solanum species*, utilized in the experiment

S. No.	Genotypes	Source	Given code	Salient features
1	*Solanum aethiopicum*	Pantnagar (AVRDC)	L_1	Erect, small, round and cluster bearing small green fruits, scarlet red while ripen, tolerant to phomopsis blight and fruit and shoot borer.
2	BARI	Bangladesh (AVRDC)	L_2	Erect plant type, light purple extra-long fruit, tolerant to phomopsis blight.
3	PB-66	Pantnagar	L_3	Erect plant type having long purple fruits.
4	Pant Rituraj (PR)	Pantnagar	L_4	Semi erect plant type, round dark purple fruit, suitable to round the year cultivation.
5	WB-1	Koochbihar (W. Bengal)	L_5	Erect and sturdy stem, oblong green fruits, tolerant to phomopsis blight.
6	PB-67(PB-6)	Pantnagar	L_6	Semi erect, long, green fruited, high yielder.
7	PB-71	Pantnagar	L_7	Erect type plant having oblong purple fruit.
8	Pant Samrat (PS)	Pantnagar	L_8	Erect type plant, long purple fruits, cluster bearing, good combiners, field resistance to bacterial wilt, tolerant to phomopsis blight and shoot and fruit borer.
9	NDB-1	NDUAT, Faizabad	L_9	Semi erect plant type, purple and oblong fruits, good combiner.
10	PB-70	Pantnagar	L_{10}	Erect type plant having round green fruit
11	PB-4	Pantnagar	L_{11}	Erect type plant, oblong purple fruits.
12	SMB-115	Cuttack	L_{12}	Erect type plant having small oblong purple fruit, cluster bearing
13	KS-331	Kalyanpur (UP)	L_{13}	Spreading plant type, long fruit having purple color
14	LC-7	Pantnagar	L_{14}	Erect, oblong, very soft and green fruits with light purple tinge.

Table 2: Concentrations of reaction mixture (25 µl/tube) for SSR primers

Components (concentration)	Final Concentration	Single tube (µl)
DNA template (50ηg/µl)	100 ηg	2.0
dNTPs mix (10mM mix)	200 µM each dATP, dCTP, dGTP, dTTP	0.5
Taq polymerase (1U/µl)	1 U	1.0
Reaction buffer (10 X)	1 X	2.5
Forward Primer (10µM)	0.6 µM	1.5
Reverse Primer (10µM)	0.6 µM	1.5
Deionised water (autoclaved)		16.0
	TOTAL	**25.0 µl**

Table 3: PCR programming for SSR primers

Cycle		Denaturation		Annealing		Polymerization	
1st cycle	x 1	94^{0}C	5:0 min	-	-	-	-
2nd cycle	x 35	94^{0}C	30 sec	45-65^{0}C	1 min	72^{0}C	1 min
3rd cycle	x 1	-	-	-	-	72^{0}C	5 min
Hold temperature at 4^{0}C							

Table 4: Mean performance of *Solanum species* genotypes in main season (kharif) 2010-11 and in ratooning during 2011 as subsequent crop

S. No.	Entry	Fruit Length (cm)			Fruit Diameter (cm)			Fruit Weight (g)			Number of Fruits/ Plant		
		Main crop	Ratoon crop	% in ratooning	Main crop	Ratoon crop	% in ratooning	Main crop	Ratoon crop	% in ratooning	Main crop	Ratoon crop	% in ratooning
1	*S. aethi.*	3.37	3.17	94.07	3.07	3.03	98.60	23.33	22.67	97.17	53.333	53.07	99.51
2	BARI	35.47	25.20	71.05	3.70	3.31	89.54	150.00	139.33	92.89	14.800	11.03	74.53
3	PB-66	16.00	14.20	88.75	5.33	4.75	89.17	141.67	139.33	98.35	16.133	15.33	95.02
4	PR	9.33	7.80	83.60	9.77	7.58	77.58	200.00	131.00	65.50	10.733	11.20	104.35
5	WB-1	8.60	11.53	134.07	6.07	4.93	81.17	124.00	94.67	76.35	13.133	9.16	69.75
6	PB-67	16.67	14.13	84.76	4.87	4.12	84.60	156.67	88.67	56.60	19.933	10.01	50.22
7	PB-71	13.27	10.87	81.91	6.60	5.25	79.50	196.67	122.00	62.03	18.400	10.35	56.25
8	PS	18.60	15.40	82.80	3.55	3.03	85.27	95.33	84.00	88.11	32.333	13.67	42.28
9	NDB-1	15.40	12.87	83.57	6.53	5.71	87.49	179.67	145.00	80.70	15.200	8.89	58.49
10	PB-70	13.67	8.43	61.67	7.27	6.76	92.98	205.33	181.67	88.48	10.000	10.40	104.00
11	PB-4	15.87	15.47	97.48	3.40	3.43	100.97	138.33	206.67	149.40	17.533	8.10	46.20
12	SMB-115	9.20z	9.20	100.00	3.13	3.12	99.62	85.00	136.67	160.79	24.867	17.87	71.86
13	KS-331	18.07	15.47	85.61	3.40	4.47	131.56	99.67	167.33	167.88	21.600	19.44	90.00
14	LC-7	17.13	11.33	66.14	8.27	8.27	100.04	236.67	251.67	106.34	13.267	5.01	37.76
	MEAN	**15.05**	**12.51**	**86.82**	**5.35**	**4.84**	**92.72**	**145.17**	**136.48**	**99.33**	**20.090**	**14.54**	**71.44**
	C D (5%)	**1.85**	**1.34**		**0.49**	**0.25**		**14.38**	**11.17**		**3.86**	**2.62**	
	S E (m)	**0.632**	**0.458**		**0.168**	**0.087**		**4.918**	**3.822**		**1.322**	**0.896**	
	C V (%)	**7.27**	**6.34**		**5.43**	**3.1**		**5.87**	**4.85**		**11.39**	**10.69**	

Note*: S. aethi.*= *S. aethiopicum*, PR= Pant Rituraj, PS= Pant Samrat

Table 4: Cont…….

S. No.	Entry	Weight of Healthy Fruits/ Plant (kg)			Weight of Infested Fruits/ Plant (kg)			Yield/ Plant (kg)			Yield (q/ha)		
		Main crop	Ratoon crop	% in ratoonin g	Main crop	Ratoon crop	% in ratoonin g	Main crop	Ratoon crop	% in ratoonin g	Main crop	Ratoon crop	% in ratoonin g
1	*S. aethi.*	0.150	0.127	84.67	0.072	0.019	26.39	0.222	0.146	65.77	49.33	32.44	65.76
2	BARI	1.443	0.898	62.23	0.500	0.305	61.00	1.943	1.203	61.91	431.85	267.40	61.92
3	PB-66	1.390	1.050	75.54	0.613	0.713	116.31	2.003	1.763	88.02	445.18	391.77	88.00
4	PR	1.263	0.712	56.37	0.537	0.564	105.03	1.800	1.276	70.89	400.00	283.48	70.87
5	WB-1	1.243	0.500	40.23	0.460	0.368	80.00	1.703	0.868	50.97	378.51	192.96	50.98
6	PB-67	1.693	0.703	41.52	0.523	0.522	99.81	2.217	1.225	55.25	492.59	272.29	55.28
7	PB-71	1.480	1.105	74.66	0.443	0.432	97.52	1.923	1.538	79.98	427.40	341.70	79.95
8	PS	1.340	0.823	61.42	0.647	0.536	82.84	1.987	1.358	68.34	441.48	301.85	68.37
9	NDB-1	1.757	0.709	40.35	0.393	0.433	110.18	2.143	1.142	53.29	476.43	253.85	53.28
10	PB-70	1.587	0.902	56.84	0.830	0.574	69.16	2.407	1.476	61.32	534.81	327.92	61.32
11	PB-4	1.453	0.722	49.69	0.597	0.514	86.10	2.050	1.236	60.29	451.81	274.59	60.78
12	SMB-115	1.477	0.706	47.80	0.383	0.683	178.33	1.860	1.389	74.68	412.59	308.66	74.81
13	KS-331	1.693	1.160	68.52	0.313	0.795	253.99	2.007	1.955	97.41	445.18	434.44	97.59
14	LC-7	1.66	0.473	28.49	0.657	0.441	67.12	2.313	0.914	39.52	513.77	203.18	39.55
	MEAN	**1.40**	**0.76**	**56.31**	**0.50**	**0.49**	**102.41**	**1.90**	**1.25**	**66.26**	**421.50**	**277.61**	**66.32**
	C D (5%)	**0.184**	**0.085**		**0.126**	**0.081**		**0.25**	**0.111**		**55.22**	**24.66**	
	S E (m)	**0.063**	**0.029**		**0.043**	**0.028**		**0.085**	**0.038**		**18.89**	**8.437**	
	C V (%)	**7.78**	**6.67**		**15.01**	**9.69**		**7.79**	**5.26**		**7.79**	**5.26**	

Note*: S. aethi.*= *S. aethiopicum,* PR= Pant Rituraj, PS= Pant Samrat

Table 5: Analysis of SSR marker

SSR PRIMERS											
S. No.	Primers code	Forward primer Sequence (5′- 3′)	Reverse primer Sequence (5′- 3′)	% GC (F)	% GC (R)	% Polymorphism	Number of bands			Unique Bands	
							Total bands	Monomorphic Bands	Polymorphic bands	'a'	'm'
1	emh11O01	GATGTGTCGATGAGATTTTGGTCA	TAGCTACGTTGGTTTGGTGCTGAA	41.60	45.80	100.00%	2	0	2	-	-
2	EMB01L13	TCAAAAGACTTGAAACCCGATGGT	GTTTATCAGGTTTTTGATCACCGGACA	41.60	40.70	100.00%	2	0	2	-	1
3	EMB01H20	TCTTGTTCCCAGTCTATCGCTAATCA	ATCCGAATTTAGTCGGGCTTCAAT	42.30	41.60	100.00%	5	0	5	1	-
4	emf21C11	TGGTTGGAGCCATGATTACTTGAA	ATGCTACCTATCAAACAGGCGGAA	41.60	45.80	100.00%	3	0	3	1	-
5	emf21H22	CACAAGATGAACAAGACTAAGGAGTGC	CTTCTTCAACCTGTCTTTAGCCCA	44.40	45.80	100.00%	3	0	3	2	-
6	EEMS15	GGGACAAATCTGACCTTTGG	CTGGTGGCAAATTCTTCGAT	50.00	45.00	00.00%	2	2	0	-	-
7	EEMS17	TGACATGTAGCTGGGCAGAG	TGGAGTGTGCATCCCAAATA	55.00	45.00	00.00%	1	1	0	-	-
8	EEMS28	GACGATGACGACGACGATAA	TGGACTCACAACTCAGCCAG	50.00	55.00	100.00%	6	0	6	1	1
9	EEMS48	CAATGCAAACAATTATCATTTCG	TCGATGTTGTTGTCGTCGTT	30.40	45.00	100.00%	6	0	6	1	1
10	EEMS49	TGAAATTGATCAATACCTATAAATTTAG	GAAAGCCAGGATAGCATTCG	21.40	50.00	100.00%	2	0	2	1	1
11	EM119	CCCCACCCCATTTGTGTTATGTT	ACCCGAGAGCTATGGAGTGTTCTG	47.80	54.10	67.00%	6	2	4	3	1
12	EM140	CCAAAACAATTTCCAGTGACTGTGC	GACCAGAATGCCCCTCAAATTAAA	44.00	41.60	86.00%	7	1	6	-	1
13	EM145	CAGTGCTACATAAATTGAGACAAGAGG	GGAGGTACAACGATTTTCATATGGT	40.70	40.00	100.00%	1	0	1	-	-
14	EM155	CAAAAGATAAAAAGCTGCCGGATG	CATGCGTGAGTTTTGGAGAGAGAG	41.60	50.00	75.00%	4	1	3	1	-
Mean/ Total				**42.31**	**46.10**	**80.57%**	**50**	**7**	**43**	**11**	**6**

'a' for *S. aethiopicum* and 'm' for *S. melongena*

Table 6: Analysis of SSR markers

S. No.	Characters	SSR
1	Total number of primers	14
2	Total number of bands	50
3	Average bands per primer	3.57
4	Minimum numbers of bands in a primer	1
5	Maximum numbers of bands in a primer	7
6	Monomorphic bands	7
7	Average monomorphic bands per primer	0.5
8	Polymorphic bands	43
9	Average polymorphic bands per primer	3.07
10	Unique bands for *S. aethiopicum*	11
11	Unique bands for *S. melongena*	6
12	Average % GC (F)	42.31 %
13	Average % GC (R)	46.10 %
14	Average % polymorphism	80.50 %
15	Number of primers exhibited 100 % polymorphism	9
16	Monomorphic primers	2

Table 7: Estimated Jaccard's similarity coefficient of 14 germplasm of *Solanum species* using SSR data

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14
L1	1.000													
L2	0.200	1.000												
L3	0.244	0.886	1.000											
L4	0.178	0.853	0.857	1.000										
L5	0.262	0.750	0.806	0.722	1.000									
L6	0.244	0.694	0.750	0.714	0.765	1.000								
L7	0.222	0.806	0.811	0.829	0.778	0.824	1.000							
L8	0.233	0.800	0.857	0.824	0.824	0.765	0.829	1.000						
L9	0.250	0.806	0.861	0.829	0.829	0.824	0.941	0.882	1.000					
L10	0.250	0.667	0.676	0.688	0.742	0.733	0.697	0.688	0.697	1.000				
L11	0.268	0.722	0.778	0.743	0.794	0.735	0.750	0.794	0.750	0.767	1.000			
L12	0.244	0.784	0.789	0.806	0.711	0.703	0.861	0.757	0.811	0.676	0.730	1.000		
L13	0.244	0.784	0.838	0.757	0.757	0.800	0.861	0.857	0.861	0.676	0.730	0.889	1.000	
L14	0.250	0.667	0.676	0.788	0.788	0.727	0.794	0.735	0.794	0.759	0.758	0.771	0.676	1.000

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-7.

Table 8: 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	3	PB-71, PS and NDB-1 (all three erect plant type)
VI	4	WB-1, PB-4, PB-70 and LC-7 (all four oblong fruits)

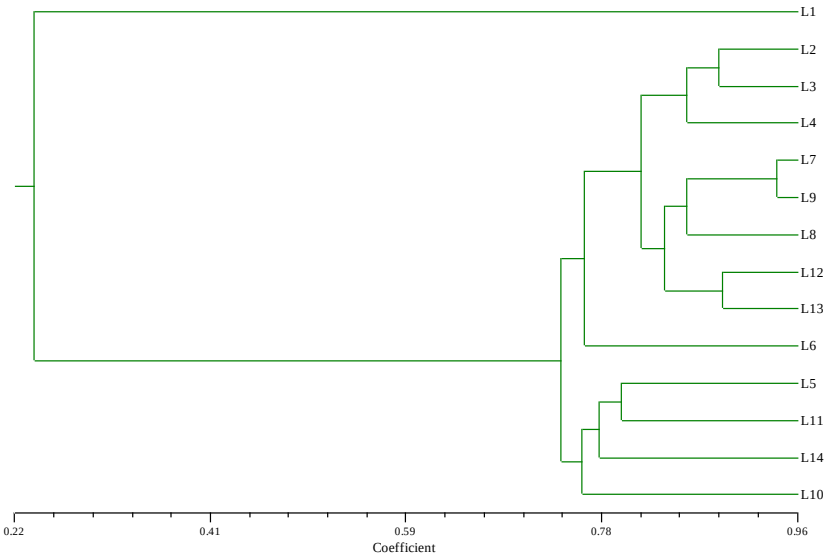

Figure 1: UPGMA dendrogram showing clustering of 14 genotypes of brinjal based on 14 SSR primers. 1=*S. aethiopicum*, *2*=BARI, 3=PB-66, 4=PR, 5=WB-1, 6=PB-67, 7=PB-71, 8=PS, 9=NDB-1, 10=PB-70, 11=PB-4, 12=SMB-115, 13=KS-331, 14=LC-7.

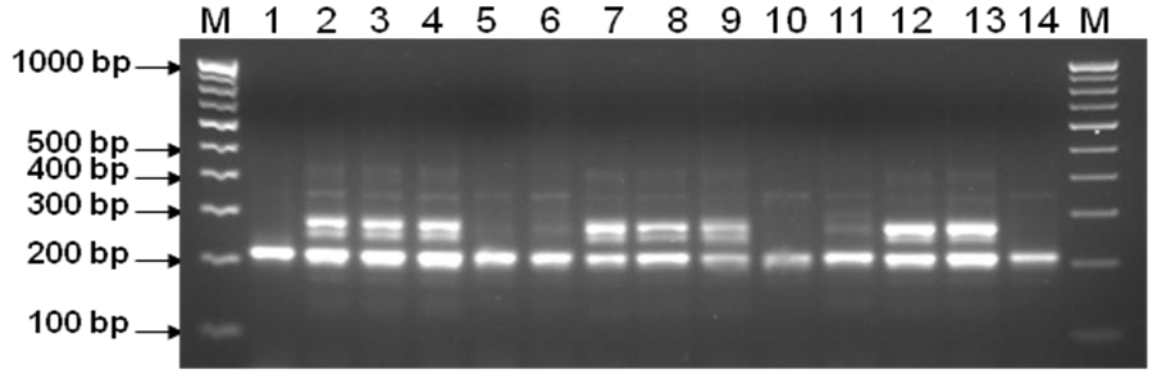

Figure 2: PCR amplification of fourteen genotypes of *Solanum species* using SSR 12 primer (EM140)

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.

Molecular analysis based on SSR markers:

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 5). Out of 50 bands, 43 bands were polymorphic (table 5). 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 6).

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 L_{12} and L_{13} and 88.6% between BARI and PB-67. The values of similarity coefficients are given in table 7.

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 8). *S. 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, PB-71, Pant Samrat and NDB-1 in cluster-V and WB-1, PB-4, PB-70 and LC-7 in cluster-VI. Morphological characters *viz.*, shape, size and peel colour of eggplant fruits showed a positive relationship with the DNA based molecular analysis through SSR marker but, not for ratooning effect.

Primer informativeness (SSR):

Out of fourteen SSR primers, primer EM119 amplified six loci with four polymorphic and three unique alleles to differentiate three different genotypes. 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. SSR primer EM140 (F:CCAAAACAATTTCCAGTGACTGTGC; R:GACCAGAATGCCCCTCAAATTAAA) differentiated brinjal genotypes in response to ratooning effect by producing double band (200 bp and 280 bp) in the genotypes BARI, PB-66, Pant Rituraj, PB-71, Pant Samrat, NDB-1, SMB-115 and KS-331 which were performing better under ratooning condition except NDB-1 (Fig. 2). The probable reason for double band might be due to heterozygocity of the parental genotypes which need selfing for at least 3 generation to confirm the double band is not a result of heterozygocity. After 3 generation selfing once again check its DNA profiling through EM140 SSR marker, double bands repeated as such then it would be concluded that this SSR is a suitable marker for ratooning effect in *Solanum* genotypes.

Molecular study has been reported by several workers like Staub and Serquen (1996), Powell *et al.* (1996), Jones *et al.* (1997), Nunome *et al.* (2003a, 2003b) and Varshney *et al.* (2005). Demir *et al.* (2010) also carried out molecular characterization of eggplant 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.

Conclusion

Yield/plant and yield/hectare were reduced in the range of 39.52 % (LC-7) to 97.40% (KS-331). Highest yield was observed in the genotype PB-70 (534.81q/ha) followed by LC-7 (513.77 q/ha) in main crop whereas, in ratoon condition it was highest in the genotype KS-331 (434.44 q/ha), which was 97.59% yield of the main crop. SAHN cluster analysis using UPGMA method separated the genotypes into six cluster groups. Morphological characters *viz.*, shape, size and peel colour of eggplant fruits and plant type showed a positive relationship with the DNA based molecular analysis through SSR markers. SSR primer EM140 produced double band in better performing genotypes under ratooning condition. Therefore, this primer could be applicable for ratooning effect in *Solanum* genotypes. Before final recommendation, it requires further investigation by selfing of genotypes for 3 generation and using more number of genotypes and primers to validate the conclusion of molecular relationship of ratooning effect among the genotypes of *Solanum* species.

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