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Assessment of Genetic Diversity in Hybrids of Tomato (*Solanum lycopersicum* L.) Using SSR Markers

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A B S T R A C T

Molecular diversity analysis on 8 hybrids of tomato generated 27 polymorphic markers. The major allele frequency for the marker SSR1-23.2 and SSR5-13.1 was least. The polymorphic information content value (PIC) ranged from 0.00 (SSR1-23.2) to 0.86 (SSR1-62.2) with an average PIC value of 0.46 per marker indicating more diversity at DNA level. Alleles ranged from 1-8 per locus with an average of 3.33 alleles per locus. Cluster analysis resulted into 3 main clusters and 4 subclusters. The diverse genotypes can be used for hybridization programmes.

Introduction

Tomato is an important and widespread vegetable in the word as fresh consumption and processed products. However, narrow genetic bases have become a bottleneck in the tomato breeding. Therefore, it is essential to know the genetic relationship between the tomato species. Molecular markers are generally recognized as a reliable means for the genetic identification among plant genotypes (Omrani *et al.*, 2007). In the past decades, all kinds of molecular markers such as restriction fragment length polymorphisma (RFLP) (Williams and Clair 1993; Messeguer *et al.*, 1991), inter-simple sequence repeat (ISSR) (Tikunov *et al.*, 2003), randomly amplified polymorphic DNA (RAPD) (Claudio *et al.*, 2004; Bernardette *et al.*, 2006), simple sequence repeat SSR (Powell *et al.*, 1996; He *et al.*, 2003; Jin *et al.*, 2004; Cooke *et al.*, 2003), and amplified length polymorphic (AFLP) (Claudio *et al.*, 2004) have been used to analyze the genetic relationships among the cultivated tomato varieties. SSR is one of the powerful DNA fingerprinting techniques. The objective of the present study was to analyze the molecular diversity of tomato hybrids.

Materials and Methods

Eight single cross hybrids were subjected for molecular diversity analysis. Thirty seven SSR

primers were used for diversity of these genotypes. The DNA from 15 days old seedlings was extracted by following mini pre-rapid method with little modifications (Doyle and Doyle, 1987). The SSR reaction mixture consisted of 25-50 ng of template DNA, 2 pM of Forward and Reverse primer, 2.5 mM of dNTPs, 1 unit of Taq polymerase (Bangalore Genei, India), 10X PCR buffer (100mM Tris pH 9.0, 500 mM KCl, 15mM MgCl2 and 0.1% Gelatin) in a volume of 10 µl. Amplification was carried out using Master Thermal Cycler 5331-Eppendorf Version 2.30, 31-09, Germany. The amplification profile was as follows.

Agarose gel of 2.5 per cent was prepared using electrophoresis grade agarose (Lonza) in electrophoresis buffer (1X TAE). After the run, the gel was viewed under UV light and the DNA banding pattern was recorded directly in UV doc.

The products of PCR were scored visually by comparing with the standard marker of size 100 bp (Bangalore Genie).

Allelic variation was calculated from the frequencies of genotypes at each locus as the polymorphic information content (PIC). Genetic parameters namely major allele frequency, genotype frequency and PIC were estimated using the software programme Power Marker version 3.25 (Liu and Muse, 2005). Dendrogram was constructed using the neighbourhood-joining algorithm using the programme DARwin 5.0 (Perrier *et al.,* 2003) and the per cent polymorphism was calculated by using the following formula,

$$\text{Per cent polymorphism} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

Results and Discussion

Thirty seven SSR primers equally spaced on different chromosomes of tomato were used for screening the 8 genotypes. Majority of the markers amplified a single allele per marker. The major allele frequency (Table 1) was least for the marker SSR1-23.2 and SSR5-13.1.

The polymorphic information content value (PIC) calculated ranged from 0.00 (SSR1-23.2) to 0.86 (SSR1-62.2) with an average PIC value of 0.46 per marker. The results revealed that out of 37 SSR primers screened, 33 markers generated amplicons in the 8 genotypes and 27 markers recorded polymorphism. Range of alleles per locus was 1-8 with an average of 3.33 alleles per locus.

The amplification profile was as follows

Sl. No.	Steps	Temperature (^{0}C)	Duration	Cycles
1	Pre-denaturation	95	5 Min	1
2	Denaturation	94	1 Min	7
3	Annealing	54-61	45 Sec	
4	Extension	72	1 Min	
5	Denaturation	94	1 Min	30
6	Annealing	54-61	45 Sec	
7	Extension	59	45 Sec	
8	Final extension	72	10 Min	1
9	Hold	4	5 Min	Until samples are removed

Table.1 Molecular diversity of number of alleles, allele frequency, gene diversity and polymorphic information content in eight tomato genotypes

SI. Number	Marker	Major Allele	Sample	Allele	Gene	PIC
		Frquency	Size	No	Diversity	
1	SSR1-0.2	0.63	8	2	0.47	0.36
2	SSR1-23.2	0.13	8	8	0.88	0.86
3	SSR1-62.2	0.13	8	8	0.88	0.86
4	SSR1-104.1	0.88	8	2	0.22	0.19
5	SSR1-153.2	0.38	8	3	0.66	0.58
6	SSR2-0.2	0.5	8	3	0.63	0.55
7	SSR2-21.1	0.38	8	3	0.66	0.58
8	SSR2-47.1	0.75	8	2	0.38	0.3
9	SSR2-79.2	0.63	8	2	0.47	0.36
10	SSR2-84.1	0.5	8	4	0.66	0.6
11	SSR2-145.1	0.88	8	2	0.22	0.19
12	SSR3-26.2	0.63	8	2	0.47	0.36
13	SSR3-61.1	0.63	8	2	0.47	0.36
14	SSR3-92.1	0.88	8	2	0.22	0.19
15	SSR3-130.2	0.75	8	2	0.38	0.3
16	SSR3-171.1	0.38	8	4	0.72	0.67
17	SSR4-10.1	1	8	1	0	0
18	SSR4-45.1	0.25	8	5	0.78	0.75
19	SSR4-67.2	1	8	1	0	0
20	SSR4-83.1	1	8	1	0	0
21	SSR4-115.1	1	8	1	0	0
22	SSR4-135.2	0.75	8	3	0.41	0.37
23	SSR5-13.1	0.25	8	7	0.84	0.82
24	SSR5-39.1	0.5	8	3	0.63	0.55
25	SSR5-69.1	1	8	1	0	0
26	SSR5-102.1	0.5	8	3	0.59	0.51
27	SSR5-112.2	0.38	8	4	0.72	0.67
28	SSR6-0.1	0.25	8	6	0.81	0.79
29	SSR6-10.1	0.25	8	6	0.81	0.79
30	TES0312	0.25	8	7	0.84	0.82
31	SSR6-17.1	0.5	8	3	0.59	0.51
32	SSR6-25.2	0.38	8	4	0.69	0.63
33	SSR6-34.1	0.5	8	3	0.59	0.51
	Mean	**0.57**	**8**	**3.33**	**0.5**	**0.46**

Table.2 Cluster distribution of eight tomato genotypes based on molecular diversity

Main cluster	Sub cluster	Number of genotypes	Cluster composition
A	I-A	3	TSH-4, TSH-5, TSH-8
	II-A	1	TSH 1
B	I-B	2	TSH-3, TSH-6
	II-B	2	TSH-2, TSH-7

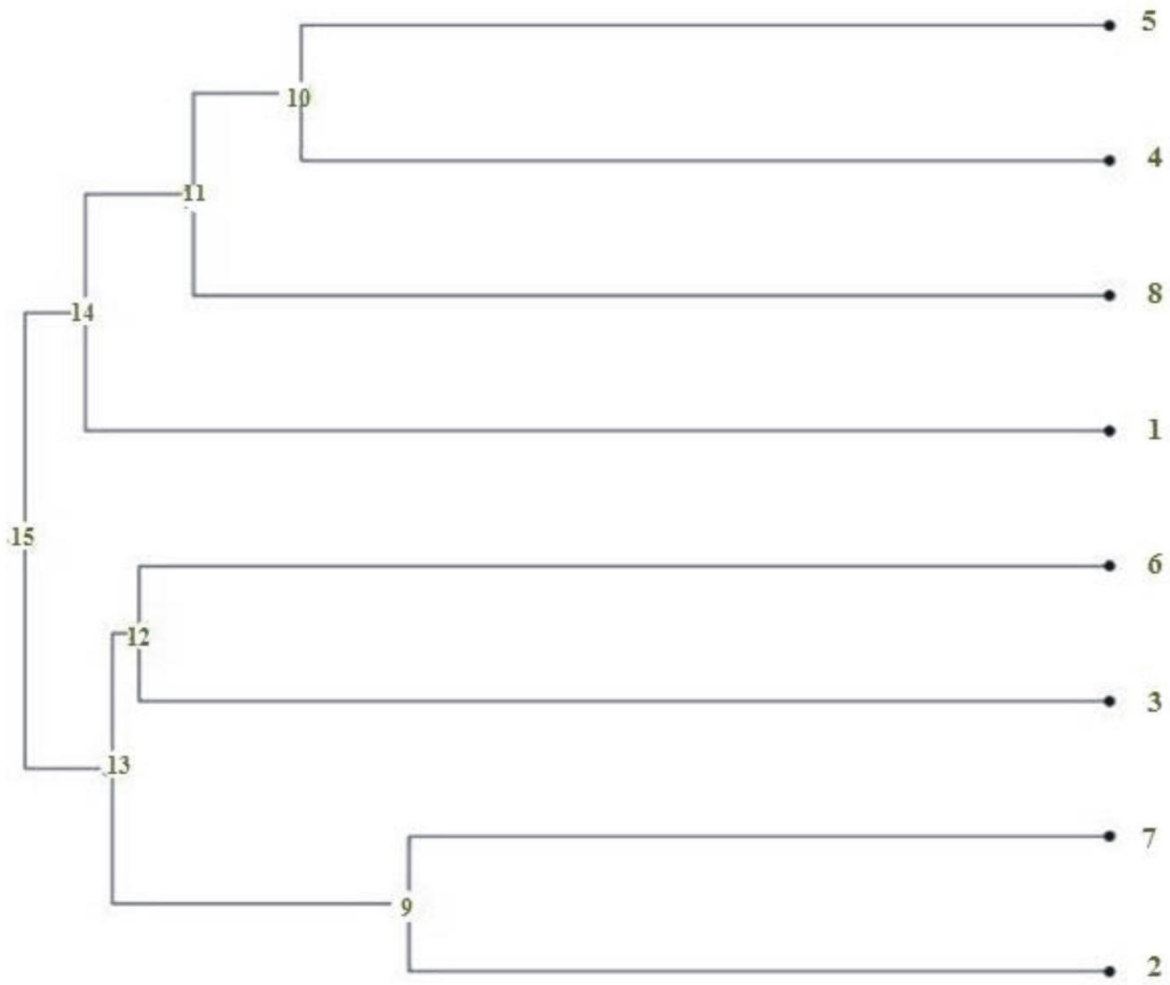

FIG 1 **Neighbour joining phenogram of eight hybrids based on genotypic data using DARwin 5.0**

The 8 genotypes were divided into three main clusters (Table 2) A and B which further formed 4 sub clusters. Among the two main clusters, Cluster A formed 2 sub clusters in which Clusters I-A had three genotypes, while Cluster II-A was solitary with one genotype in it. Main Cluster B had 2 sub clusters and among them, Cluster I-B and Cluster II-B had two genotypes each having two genotypes (Fig. 1).

Range of 1-8 alleles per locus with an average of 3.3 alleles per locus was observed. Similar PIC value range, allele number and allelic frequency in tomato were reported by Ezekiel *et al.,* (2011) and Saida *et al.,* (2013) with various markers. The results of hierarchical clustering in this study grouped the accessions into two main clusters, 'A' and 'B' and these were divided into four sub-clusters. However, the cluster analysis in the present study indicated wide range of variability at genotypic level. The polymorphism at genotypic level can be assessed by generating high polymorphic markers. High resolution of markers is needed for characterization and evaluation which can be done by increasing the number of repeat units and length of the marker. When compared with clusters formed through D^2 analysis no single genotype was common between them indicating that phenotypic diversity is not associated with genotypic diversity.

References

Bernardette, P. C., Gerald, L. T. S., Grazziotin, F. G. and Echeverrigaray, S., 2006. Genetic diversity among Brazilian cultivars and landraces of tomato *Lycopersicon esculentum* Mill. Revealed by RAPD markers. Genetic Resources and Crop Evolution. 53, 395-400.

Claudio, D. G., Pasqua, D. O., Angela, B., Franco, C., Concetta, L. and Luigi, R., 2004. Identification of PCR-based markers (RAPD, AFLP) linked to a novel powdery mildew resistance gene (0l-2) in tomato. Plant Science. 166, 41-48.

Cooke R J, Bredemeijer G M M, Ganal M W, Peters R, Isaac P, Rendell S, Jackson J, Röder M S, Korzun V, Wendehake K, *et al.,* 2003. Assessment of the uniformity of wheat and tomato varieties at DNA Microsatellite loci. *Euphytica*, 132, 331-341.

Doyle, J. J. and Doyle, J. L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin. 19: 11-15.

Ezekiel, C. N., Nwangburuka, C. C., Ajibade, O. A. and Odebode, A. C., 2011. Genetic diversity in 14 tomato (*Lycopersicon esculentum* Mill.) varieties in Nigerian markets by RAPD-PCR technique. African J. Biotechnol. 10(25): 4961-4967.

He, C., Poysa, V. and Yu K., 2003. Development and characterization of simple sequence repeat (SSR) markers and their use indetermining relationships among *Lycopersicon esculentum* cultivars. Theoretical and Applied Genetics. 106, 363-373.

Jin, F. M., Xue, J., Xia, S. Y. and Liu, Z. Q., 2004. Application of SSR marker technique to the tomato genetic breeding. Tianjin Agricultural Sciences. 4, 13-17. (in Chinese)

Liu, K. and Muse, S. V., 2005. Powermarker: Integrated analysis environment for genetic marker data. Bioinform. 21: 2128-2129.

Messeguert, R., Ganal, M., de Vicente, M. C., Young, N. D., Bolkan, H. and Tanksley, S. D., 1991. High resolution RFLP map around the root knot nematode resistance gene (*Mi*) in tomato. Theoretical and Applied Genetics. 82, 529-536.

Perrier, X., Flori, A. and Bonnot, F., 2003. Data analysis methods, Science Publishers, Montpellier, pp. 43-76.

Powell, W., Machray, G. C. and Provan, J., 1996. Polymorphism revealed by simple sequence repeats. Trends Plant Science. 1: 215- 222.

Saida, S., Sabina, M., Konstantinos, T. and Konstantinos, R., 2013. Assessment of genetic diversity in cultivated tomato (*Solanum lycopersicum* L.) genotypes using RAPD primers. J. of Horticultural Research. 21(1): 83-89.

Tikunov, Y. M., Khrustaleva, L. I. and Karlov, G. I., 2003. Application of ISSR markers in the genus *Lycopersicon*. Euphytica. 131, 71- 80.

Williams, C. E. and Clair, D. A. S., 1993. Phenetic relationships and levels of variability detected by restriction fragment length polymorphism and random amplified polymorphic DNA analysis of cultivated and wild accessions of *Lycopersicon esculentum.* Genome. 36, 619-630.

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