Indian J. Genet., 69(1): 44-49 (2009)

Detection of genotype specific fingerprints and assessment of genetic diversity in elite genetic stocks of tomato (*Solanum lycopersicum* L.) using RAPD primers and agronomic traits

M. S. Dhaliwal[1,3], Sawtantar Singh[1] and Kuldeep Singh[2]

[1]Deptt. of Vegetable Crops, [2]School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana 141 004

(Received: January 2008; Revised: December 2008; Accepted: January 2009)

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

Abstract

Genotype specific DNA fingerprints and morphological traits for genetic stocks of crop plants are pre-requisite for germplasm registration with the competent authority and for granting Plant Breeders' Rights. Eight morphological and three biochemical markers, and 18 RAPD primers were employed to estimate genetic diversity and to characterize 30 genetic stocks of tomato possessing special attributes. The analysis based on field observations, biochemical constituents and RAPD primers revealed wide genetic diversity in the germplasm evaluated. The RAPD primers generated 192 bands of which, 151 (78.6%) were polymorphic. The polymorphic information content (PIC) values for the 18 primers ranged from 0.76 in OPB-154 to 0.97 in S-1113. The similarity co-efficient analysis revealed two clusters; the first cluster comprised of only four genotypes and the second major cluster comprised of 26 genotypes which could further be classified in six sub-clusters. The RAPD analysis proved helpful for estimating the magnitude of genetic diversity, establishing genetic relatedness among genetic stocks and for developing unique fingerprints of 21 out of 30 genetic stocks evaluated.

Key words: Tomato, molecular markers, RAPDs, fingerprinting, genetic diversity

Introduction

Diverse germplasm including specific genetic stocks are the most valuable basic materials for crop breeders to meet the current and future needs. Characterization of genetic stocks and varieties is mandatory for the purpose of registration with the competent authority and for granting Plant Breeders' Rights under the criteria of distinctness, uniformity and stability (DUS). Characterization of tomato (*Solanum lycopersicum* L.) varieties/genotypes using morphological markers requires collection of extensive field data. Using morphological markers, it is easier to characterize the germplasm at the species level, but identification of genotypes within a species based on morphological markers alone is relatively difficult. Among molecular markers currently employed, random amplified polymorphic DNA (RAPD) markers are cost effective and do not require any prior information of the genome [1]. Optimization of PCR conditions, following same protocol for each of the repeat tests and scoring only reproducible bands improves efficiency of RAPDs analysis [2].

In tomato, various molecular markers *viz.*, isozymes, RFLPs, RAPDs, AFLPs and SSRs have been used to assess genetic diversity in germplasm collections [3-8] and for germplasm characterization [9-10]. The present investigation was undertaken with the objectives of estimation of genetic diversity and characterization of important genetic stocks of tomato using morphological, biochemical and RAPD markers.

Materials and methods

Experimental material

The experimental material comprising 30 genotypes included important tomato genetic stocks such as male sterile lines (both pollen abortive and functional male sterile types), high TSS and high lycopene lines; nematode, leaf curl virus, early blight, late blight, *Fusarium* wilt and *Verticillium* wilt resistant lines; non-ripening mutants and the ones possessing abiotic stress tolerance. The lines *viz.*, L 3707 and L 3708 belongs to the wild species *L. pimpinellifolium* and rest of the genotypes belongs to the cultivated species. The horticultural traits evaluated included days to flowering, days to fruit set, fruit weight (g), polar diameter (cm), equatorial diameter (cm), fruit shape index (ratio of polar to equatorial diameter), number of locules, pericarp

[2]Corresponding author; [3]Present address: AVRDC-The World Vegetable Center, C/o ICRAF, PO Box 16317, Yaounde, Cameroon

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

thickness (cm), total soluble solids (TSS %) (using hand refractometer), acidity (g 100^{-1}ml of juice) [11] and lycopene content (mg100^{-1}g fresh fruit) [12].

Genomic DNA extraction and RAPD analysis

The genomic DNA was extracted from fresh leaf tissues following CTAB method as described by Saghai-Maroof *et al.* [13]. Quantity and quality of DNA was checked both by gel electrophoresis and spectrophotometer. Genomic DNA was amplified through PCR using RAPD primers in an Eppendorf Master Cycler™. Initially 25 RAPD primers were used for amplification of a set of five diverse genotypes. Eighteen RAPD primers that showed good amplification in the representative set were later used for amplification of whole set of genotypes. The RAPD allele sizes were determined based on the position of bands relative to the ladder (Fermantas Gene Ruler 1KB DNA lader). Total number of alleles was recorded for each of the 18 RAPD primers in all the 30 genotypes by assigning allele numbers as 1, 2, 3, 4 and so on. With each primer, the band having highest molecular weight was designated as allele 1. The amplified bands in the whole germplasm set were recorded in a binary matrix as 1 (band present) or 0 (band absent). The polymorphic information content (PIC) values for each of 18 primers were estimated using the formula:

$$PIC = 1 - \sum_{j=1}^{n} P_{ij}^{2}$$

where P_{ij} is the frequency of jth allele in the ith primer.

Cluster analysis

The RAPD marker amplification profile of thirty genotypes was used to estimate genetic diversity/relatedness based on number of shared amplified bands. The presence or absence of a particular amplification product was used as an index of genetic diversity/relatedness. The similarity matrix value based on Jaccard [14] coefficient of similarity was used to generate dendrogram. Clustering was done by UPGMA using SHAN module of NTSYSpc.Version 2.02e [15].

Results and discussion

Evaluation of tomato genetic stocks for morphological and biochemical attributes

The analysis of variance for the characters evaluated revealed that the mean squares due to genotypes were highly significant for all the traits (data not shown). The mean values for various characters in 30 genotypes along with extent of diversity (range) and their respective LSD values are reported in Table 1. Minimum values for days to flowering, days to fruit set, fruit weight and pericarp thickness were recorded in L 3708, a *L. pimpinellifolium* genotype. Fruit shape index as determined by the ratio of polar to equatorial diameter varied from 0.63 in Edkawi to 1.90 in Punjab Chhuhara. Highest fruit weight, maximum number of locules and thickest pericarp were recorded in *ms 45* VFN 8, LA 1502 and RM-2, respectively. Among quality attributes, the highest value for TSS, acidity and lycopene content were observed in LA 1501, *rin* T3 and *ms 2* IPA-3, respectively. Except for lycopene content that varied from 0.00 in *rin* T3 and RM-2 to 3.84 mg^{-1}100g in *ms 2* IPA-3, a wide range in phenotypic means of both, morphological and biochemical characters was revealed. Earlier, Hanson *et al.* [16] evaluated 53 tomato lines belonging to *L. esculentum* and *L. pimpinellifolium* and reported that lycopene content varied from 0.04 mg^{-1}100g in LA 2997 to 23.09 mg^{-1}100g in LA 1582. Since, diversity between the parents is an important factor in determining extent of improvement; the present investigation revealed that there exists a tremendous scope for tomato genetic improvement through hybridization for most of the characters evaluated.

RAPD analysis

Of the 25 primers screened, seven primers *viz.*, S1109, S1114, S1102, S1120, S1106, S1108 and S112 (Q-Biogene) amplified few bands, but the bands were faint and not scorable unambiguously. On the basis of easily scorable amplified bands, 18 primers were selected for final analysis. The primers used for genotyping the tomato genetic stocks along with their base sequence, total number of amplified bands and number of polymorphic bands generated by each of the primers is listed in Table 2. The number of bands amplified was primer and genotype dependent and ranged from 7 in S1103 and S1111 to 16 in OPA 175 and S1113. A total of 192 bands were amplified with 18 primers with an average of 10.6 bands per primer. Out of 192 bands amplified, 151 were polymorphic (78.6%) with an average of 8.4 bands per primer and 41 (21.4%) were monomorphic with an average of 2.2 bands per primer. Earlier, Williams and St-Clair [4], Shekara *et al.* [6] and Noli *et al.* [10] also used RAPD primers for characterizing cultivated and wild species of tomato. Williams and St-Clair [4] reported that the 46 accessions evaluated were monomorphic at 135 of the 215 RAPD loci assayed. Using RAPD primers, Noli *et al.* [10] distinguished the cultivated types of tomato from its wild relatives. Shekara *et al* [6] characterized four species of tomato using

Table 1. Mean performance of genetic stocks of tomato for various horticultural traits and biochemical constituents

S.No.	Genotype	Days to flowering	Days to fruit set	Fruit weight (g)	Polar dia. (cm)	Equatorial dia. (cm)	Fruit shape index	No. of locules	Pericarp thickness (cm)	Total soluble solids (%)	Acidity (g^{-1} 100 ml of juice)	Lycopene (mg^{-1} 100g)
1.	*ms-10*[36] VF 36	22.6	76.6	131.6	5.1	7.1	0.78	5.0	0.43	4.56	0.59	0.79
2.	*ms-45* VFN 8	27.0	60.3	132.3	4.9	7.3	0.82	7.0	0.53	4.53	0.42	0.55
3.	*ms-16* Pritchard	48.6	84.6	101.0	4.7	5.9	0.81	4.6	0.50	4.63	0.87	1.40
4.	PNR-7	42.0	62.3	73.1	3.9	5.3	0.75	7.0	0.43	4.53	0.62	0.77
5.	Edkawi	59.3	72.6	76.1	3.8	5.9	0.63	8.0	0.36	3.36	0.51	0.28
6.	RM-2	36.3	66.6	72.8	5.7	5.2	1.00	2.0	0.86	4.60	0.75	0.00
7.	EBR-6	37.3	80.0	61.1	6.0	5.7	1.20	3.0	0.46	4.93	0.59	0.50
8.	Nemadoro	29.3	50.6	71.8	5.4	4.7	1.20	3.0	0.83	3.86	0.52	2.50
9.	SanPedro	36.6	58.3	73.0	4.7	5.0	0.93	3.3	0.63	4.86	0.52	2.42
10.	Punjab Chhuhara	49.6	73.0	48.3	6.6	3.3	1.90	3.0	0.56	5.16	0.64	1.80
11.	Healani	29.6	56.6	63.3	4.0	4.8	0.83	6.3	0.43	3.13	0.63	0.75
12.	L 3841	30.0	51.6	63.0	4.5	4.9	0.87	3.6	0.56	4.90	0.61	1.53
13.	WIR 4285	41.0	54.6	70.0	4.3	4.5	0.98	4.3	0.30	4.43	0.85	1.27
14.	IPA 3	38.3	60.6	77.0	3.7	3.2	1.10	2.3	0.60	4.70	0.63	1.28
15.	F_2 A 3-1-3-5	32.0	56.6	30.3	3.5	3.7	0.95	2.0	0.36	4.43	0.75	2.66
16.	F_2 A 3-3-1-1	42.6	64.6	22.8	3.8	3.2	1.10	2.0	0.43	3.90	0.84	1.00
17.	F_2 B 4-3-3	59.3	85.3	32.5	3.7	3.6	1.02	2.0	0.40	4.50	0.63	3.00
18.	F_2 B 13-4-1	36.0	51.6	29.5	3.7	3.6	1.02	2.3	0.36	4.03	0.62	1.59
19.	F_3 C 2-12-1	46.0	54.6	34.6	3.9	3.8	1.00	3.3	0.46	4.60	0.52	1.28
20.	ps_2 L 3841	36.0	70.3	38.6	3.5	4.0	0.87	4.0	0.23	4.83	0.63	3.17
21.	ps_2 NS 101	33.6	76.6	59.3	4.6	4.6	0.98	3.0	0.46	4.83	0.70	3.30
22.	LA 1500	51.3	79.0	92.0	4.7	5.6	0.81	6.6	0.46	5.96	0.83	0.70
23.	LA 1501	48.3	80.6	105.0	4.3	5.8	0.92	7.3	0.36	7.60	0.49	1.15
24.	LA 1502	60.0	80.6	130.0	4.7	7.1	0.64	8.6	0.46	6.10	0.56	1.14
25.	L 3707	29.6	85.6	3.4	1.7	1.6	0.93	2.0	0.16	4.60	1.00	1.07
26.	L 3708	16.3	28.6	1.8	1.1	1.1	0.93	2.0	0.10	5.10	0.83	1.41
27.	8-2-1-2-5	46.6	92.3	55.1	6.3	4.1	1.50	2.3	0.63	4.90	0.49	2.81
28.	*rin* T-3	34.0	55.3	95.8	4.6	6.4	0.72	6.0	0.43	4.70	1.20	0.00
29.	*ip*NILVFN 145	65.0	97.3	106.0	6.4	5.5	1.14	4.0	0.66	4.06	0.85	0.38
30.	*ms2* IPA-3	45.3	68.3	72.0	4.6	5.1	0.89	2.6	0.56	4.56	0.71	3.84
Range		16.3-65.0	28.6-97.3	1.8-132.3	1.1-6.7	1.1-7.3	0.63-1.90	2.0-8.6	0.10-0.80	3.10-7.60	0.42-1.20	0.00-3.84
CD at p = 0.05		3.15	2.99	2.63	0.20	0.21	0.07	0.86	0.08	0.21	0.02	0.18
CD at p = 0.01		4.13	3.92	3.45	0.27	0.28	0.09	1.13	0.11	0.28	0.03	0.23

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

RAPD markers. They obtained a total of 77 amplification products where 61 were polymorphic with an average of 6.42 bands per primer.

The PIC values for the 18 primers ranged from 0.76 in primer OPB-154 to 0.97 in primer S1113 with an average of 0.87 for all 18 primers (Table 2). Primer OPA-175 amplified a total of 16 bands in 30 genotypes with PIC values of 0.94, whereas primer S1118 amplified only 10 bands with PIC value of 0.96. Thus in the present set of genotypes, primer S1118 was more informative than primer OPA-175. The PIC values being high, thus the set of primers used was informative.

Cluster analysis

The genetic relationships among the genotypes are presented in the form of dendrogram (Fig. 1). At 50% similarity level, the dendrogram revealed two clusters. The first cluster comprised only four genotypes and the second major cluster comprised 26 genotypes. The first cluster is represented by three genotypes from USA and one from Punjab (India) at overall similarity coefficient of 48%. The genotypes *ms10*[36] VF 36 and *ms45* VFN8 were clustered into one sub-group at

Table 2. Random amplified polymorphic DNA (RAPD) primers used for genotyping of important tomato genetic stocks

S.No.	Primer designation	Primer sequence	Total no. of bands	No. of poly-morphic bands	PIC value
1.	S1118	CCAGGTCTTC	10	6	0.96
2.	S117	CACTCTCCTC	10	8	0.91
3.	S1115	GATGCGATGG	13	10	0.94
4.	S1112	TCTCACCGTC	9	6	0.86
5.	S1116	TGGCGGTTTG	9	7	0.85
6.	S1111	AGATGCGCGG	7	4	0.84
7.	S1119	CCAGGTCTTC	12	8	0.90
8.	S1107	AACCGCGGCA	12	11	0.85
9.	S1113	CACGGCACAA	16	10	0.97
10.	S1103	CTTCCCTGTG	7	7	0.81
11.	S106	ACGCATCGCA	11	9	0.88
12.	S109	TGTAGCTGGG	10	10	0.83
13.	S1117	GCTAACGTCC	11	8	0.89
14.	OPA-175	TCGCGTGACT	16	13	0.94
15.	OPC-15	GACGGATCAG	11	7	0.90
16.	OPA-18	AGGTGACCGT	9	8	0.77
17.	OPB-154	CGATACGACG	10	10	0.76
18.	OPE 08	TCACCACGGT	9	9	0.81
TOTAL			192	151	

similarity coefficient of 68%. Genotype *ms2*IPA-3 was developed at PAU by incorporating *ms2* gene from *ms2* Pearson through backcrossing. Both IPA-3 and *ms2* Pearson were originally introduced from the USA. Thus VF 36 and VFN 8 having different ms genes *ms10* and *ms45* still show considerable similarity with IPA-3. Genetic stock *ms2* IPA-3 developed through backcrossing shows considerable genetic diversity with the recurrent parent IPA-3 that clustered in a different group (IId). This indicated the need to make few more back crosses to recover genotype of the recipient parent.

The major cluster having 26 genotypes included germplasm from different regions *viz*., 11 from Punjab (India), 10 from USA, three from Taiwan and one each from Russia and France. Group II is further sub-clustered in six groups, although all these exhibited an overall similarity of about 48%. Tomato leaf curl virus (ToLCV) resistant stocks *viz*., F_2A 3-1-3-5, F_2A 3-3-1-1, F_2B 4-3-3, F_2B 13-4-1 and F_3C 2-12-1 developed from the same cross, clustered into one sub group (IIc) at overall similarity of more than 70%. The functional male sterile lines *viz*., ps_2 L3841 and ps_2 NS 101 clustered in one

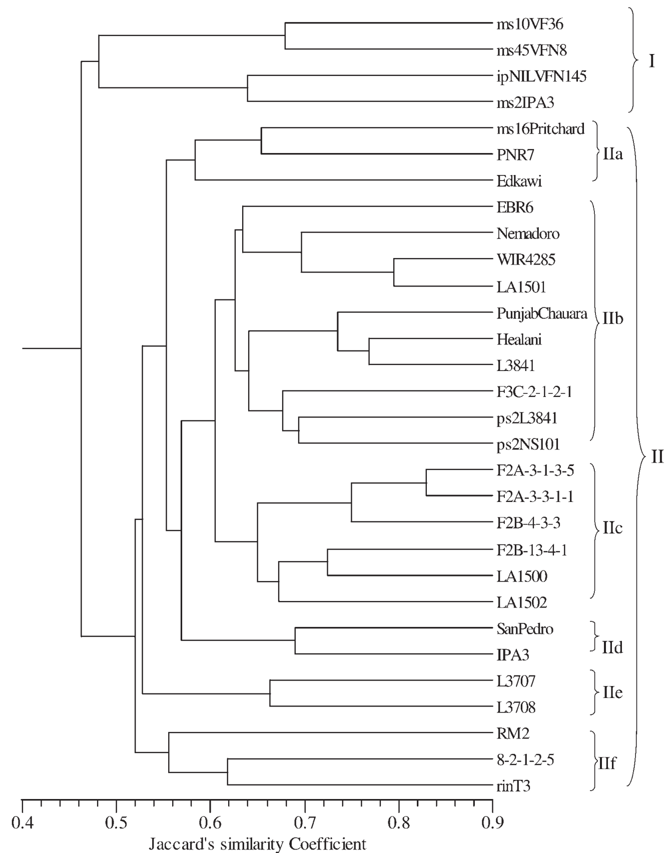

Fig. 1. UPGMA based dendrogram exhibiting genetic relationships in a set of tomato genetic stocks using RAPD markers

sub-group (IIb) at 69% similarity. In both the genotypes the functional male sterile gene 'ps_2' was introduced from the same donor through conventional back-cross breeding method. The processing types San Pedro from France and IPA-3 from USA clustered into one sub-group (IId) at 69% similarity. The genotypes LA 1500 and LA 1502, introduced from the USA and having high total soluble solid content, clustered into one sub-group (IIc) with 67% similarity coefficient. The non-ripening mutants RM-2 from Punjab, India and *rin* T3 from the USA also clustered in one sub group (IIf) with 56% similarity level. RAPD markers clearly distinguished the small fruited genotypes L 3707 and L 3708, both belonging to wild species *L. pimpinellifolium.* These genotypes formed one sub-group (IIe) at 66% similarity coefficient. Thus horticultural relatedness in these genotypes was revealed at the DNA level.

It was further revealed that the major group included the genotypes both from indigenous and the exotic sources. This indicated that the geographic distribution may not be the true index of genetic diversity in tomato. This could be attributed to the fact that so far genetic resources have been freely exchanged all over the world and were exploited for crop improvement programmes. Further, recent breeding trends towards a specific plant and fruit type seems to have contributed considerably to genetic uniformity among the modern cultivars.

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

Genotype fingerprints

The 18 RAPD primers were used for *in vitro* amplification of the 30 genotypes. The results revealed that each primer exhibited a specific banding pattern and it was possible to differentiate 21 of the 30 genotypes used in the present study (Table 3). The remaining nine genotypes could not be distinguished on the basis of RAPD analysis. There were many alleles which were specific to few genotypes. Primer E 08, for example, amplified nine scorable bands and one band (500-750bp) was amplified in all the 30 genotypes (Fig. 2) except for # 21, which might be a pippetting error. This primer unequivocally differentiated all the 29 genotypes in seven groups. Genotypes 13, 22, 25 and 26 had unique fingerprints, and genotypes 6 and 28 had distinct but similar fingerprint. Remaining 24 genotypes formed three groups comprising genotypes 1, 2, 3, 18, 19, 20, 23; 4, 5, 7, 9, 11, 12, 14, 15, 16, 17, 24, 29, 30 and 8, 10, 27. Primer S 1107 could differentiate genotypes 6 and 28, genotype 2 from 1 and 3 and genotypes 8 and 27 from 9 and 10.

Table 3. RAPD primers that could fingerprint tomato genotypes uniquely

S. No.	Genotype	Distinguish primers
1.	*ms-10*^{36} VF 36	S 1112, S 1119, S 1116
2.	*ms-45* VFN 8	S 1112, S 1107, S 1119, S 1115
3.	*ms-16* Pritchard	S 1119, OPA-18
4.	PNR-7	OPB 154, S 106, S 1119, OPA-18
5.	Edkawi	OPC-15, OPA-18
6.	RM-2	OPE 08, OPB 154, S 1118, S 1112, S 1107, OPA-175
7.	EBR-6	S 1107
8.	San Pedro	S 1107
9.	L 3841	S 1112
10.	F_2A 3-1-3-5	OPB 154, S 1117
11.	F_2A 3-3-1-1	OPB-154, S 1117
12.	F_3C 2-12-1	S 109
13.	*ps*$_2$ L3841	OPE 08
14.	*ps*$_2$ NS 101	S 1107
15.	LA 1502	S 106
16.	L 3707	OPB 154, S 1117, S 109, S 1116, OPA-175, S 1111
17.	L 3708	OPE 08, OPB 154, S 1117, S 109, S 1107, S 1116, S 1111, S 117
18.	8-2-1-2-5	OPB 154, S 109, S 1107, S 1116, OPC 15
19.	*rin* T-3	OPE-08, OPB 154, S 1118, S 109, S 1107, S 1116, OPA 175, S 1111
20.	*ip*NILVFN-145	OPB 154, S 1103, S 1112, S 1119, S 1116, S 1115, OPA-18
21.	*ms*2 IPA-3	S 106, S 1103, S 1119, S 1116, OPA-175, S 117, OPA-18

The primers OPE 08, OPB 154, S 1118, S 1107, and OPA-175 differentiated both the non-ripening mutants RM2 and *rin* T3 from other genotypes. The primer OPB 154 and S 1117 amplified bands that were specific to the two virus resistant genotypes F_2A 3-1-3-5 and F_2A 3-3-1-1 and could fingerprint these genotypes uniquely. Three primers, S 1119, S 1115, and OPA-18 could differentiate male sterile lines viz. *ms10*^{36} VF 36, *ms45* VFN8 and *ms16* Pritchard. The primer S 1107 amplified bands specific to genotypes namely EBR-6, San Pedro and *ps2* NS 101. Another four primers namely S1116, S1111, OPA-175 and S1117 could differentiate the wild tomato genotypes *viz*., L 3707 and L 3708 from the cultivated types

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

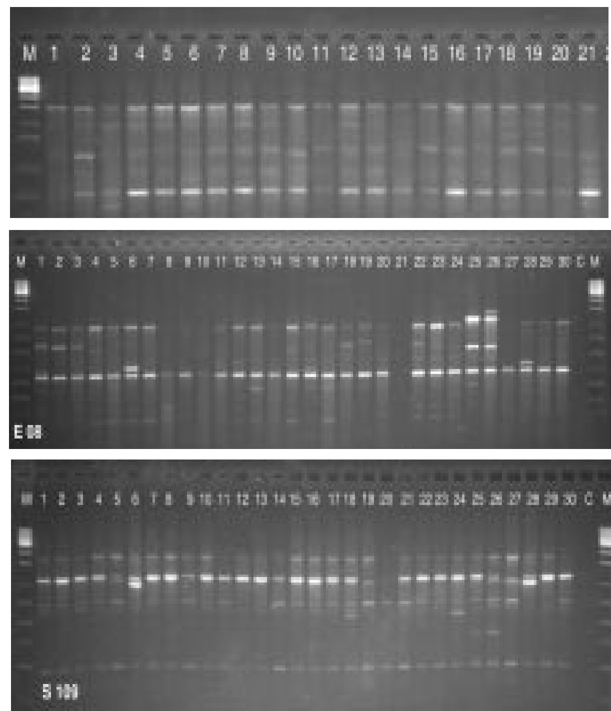

Fig. 2. RAPD amplification profile of 30 tomato genetic stocks. M = marker 1Kb ladder; C = Nagative control; Lanes 1-30 are tomato genotypes

It is concluded that, except for lycopene content, there existed a wide range of variation for other morphological and biochemical characters evaluated. RAPD analysis proved helpful for estimating the magnitude of genetic diversity at molecular level and establishing genetic relatedness among genetic stocks evaluated. Cluster analysis revealed that the genotypes were not grouped as per their geographic distributions. The recent trends toward breeding for a specific plant and fruit type seem to have brought about considerable genetic uniformity among the modern cultivars. On the basis of banding pattern, RAPDs were effectively used for molecular characterization of tomato genetic stocks used in this study.

References

1. **Williams J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A. and Tingey S. V**. 1990. DNA Polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, **18**: 6531-6535.
2. **Yonemoto Y., Chowdhury A. K., Koto H. and Macha M. M**. 2006. Cultivars identification and their genetic relationships in *Dimocarpus longan* subspecies based on RAPD markers. Scientia Horticulturae, **109**: 147-152.
3. **Miller J. C. and Tanksley S. D.** 1990. RFLP analysis of phytogenetic relationships and genetic variation in the genus *Lycopersicon.* Theor. and Appl. Genet., **80**: 437-448.
4. **Williams C. E. and St-Clair D. A**. 1993. Phenetic relationship 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.
5. **Archak S., Karihaloo J. L. and Jain A**. 2002. RAPD markers reveal narrowing genetic base of Indian tomato cultivars. Current Science, **82**: 1139-1143.
6. **Shekara A. C. C., Kulkarni R. S. and Muniyappa V**. 2003. Genetic distance of four species of tomato as revealed by RAPD markers. Indian J. Genet., **63**: 275-276.
7. **Zhu H., Zhang H., Mao K. M., Li. H. T. and Wang A**. 2004. The genetic diversity of Yunnan local varieties and wild species of tomato. J. Yunnan Agricultural University, **19**: 373-377.
8. **Park Y. H., West M. A. L. and St-Clair D. A**. 2004. Evaluation of AFLPs for germplasm fingerprinting and assessment of genetic diversity in cultivars of tomato. Genome, **47**: 510-518.
9. **Smulders M. J. M., Bredemeijer G., Rus-Kortekaas W., Arens P. and Vosman B**. 1997. Use of short microsatellites from database sequences to generate polymorphism among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. Theor. Appl. Genet., **97**: 264-272.
10. **Noli E., Conti S., Maccaferri M. and Sanguineti M. C**. 1999. Molecular characterization of tomato cultivars. Seed Science & Technology, **27**: 1-10.
11. **Anon**. 1951. Sampling techniques and chemical analysis of vegetables. Southern Co-operative Society Bulletin, **10**: pp. 134.
12. **Ranganna S.** 1994. Handbook of analysis and quality control for fruit and vegetable products. 2nd Ed. Tata McGraw-Hill Co. Ltd, New Delhi, India.
13. **Saghai-Maroof M. A., Soliman K. M., Jorgensen A. R. and Allard R. W.** 1984. Ribosomal DNA spacer length molymorphism in barley: inheritance, chromosomal location and population dyanamics. Proc. Nat. Acad. Sci., USA, **81**: 8014-8018.
14. **Jaccard P**. 1908. Nouvelles researches Sur la distribution florale. Bull. Soc. Vaud Sci. Nat., **44**: 233-270.
15. **Rohlf F. J.** 1998. NTSYS-PC Numerical Taxonomy and Multivariate System, Version 2.0. Applied Biostatis Inc., New York.
16. **Hanson M. P., Ray-yu Y., Jane W., Jen-tzu C., Dolores L. and Samson C. S. T.** 2004. Variation for antioxidant activity and antioxidants in tomato. J. American Soc. Horticultural Science, **129**: 704-711.