



ASSESSMENT OF GENETIC VARIATION IN TOMATO (*Solanum Lycopersicum L.*) BASED ON QUALITY TRAITS AND MOLECULAR MARKERS

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SUMMARY

The demand of tomato and its products continue to rise as it is an excellent source of antioxidant nutrients. The present study was carried out to study the phylogenetic relationships of 10 selected tomato genotypes using random amplified polymorphic DNA analysis. Significant differences ($P \leq 0.05$) were observed among the tomato lines for the principal antioxidants phytonutrients, viz. total carotenoids, lycopene and vitamin C. Vitamin C content ranged from 15.82-31.93 mg/100 g in fresh weight, the total carotenoid content ranged from 4.92-7.66 mg/100 g, and lycopene content ranged from 3.33-5.66 mg/100 g. Significant variation ($P \leq 0.05$) was also observed for pH and anhydrous citric acid (acidity). The pH varied from 3.70-4.46 and anhydrous citric acid ranged from 0.267-0.56%. The total soluble solids varied from 2.50-4.66%. The maximum Vitamin C content, Acidity was recorded in 2012/TOMATO Hyb DET AVT-3 (31.93 mg/100 g) whereas maximum total carotenoid content were recorded in 2012/TOMQTO AVT DET-3 (7.66 mg/100 g). Maximum lycopene content was estimated in 2012/TOMQTO AVT DET-8 (5.68 mg/100 g). Out of 10 primers screened, only four random primers gave reproducible polymorphic DNA bands. A total number of 35 amplified DNA bands were generated across the studied line with average of 8.75 bands/primer. Out of 35 bands, 26 bands were polymorphic. Cluster analysis based on UPGMA divided the tomato lines into 3 distinct clusters. In cluster I five tomato lines, in cluster II two tomato lines and in cluster III three tomato lines were observed. It could be concluded that, RAPD markers are important for genetic analysis and indicate a considerable amount of genetic diversity between the different studied varieties of tomato lines.

Key words: Genetic diversity, antioxidant, carotenoid, lycopene, RAPD, cluster analysis, *Lycopersicon esculentum L.*

Key findings: In this study significant variability was observed among the tomato based on quality traits as well as molecular data. Molecular analysis using RAPD markers was effective in assessing and discriminating the tomato lines.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a member of family Solanaceae and vegetable crop of special economic importance in the horticultural industry worldwide (He *et al.*, 2003 and Wang *et al.*, 2005). Although the genus *Lycopersicon* includes a few species, its taxonomy is still questionable and phylogeny has not been completely established (Warnock, 1988). The popularity of tomato and its products continue to rise as it is a good source of antioxidant nutrients.

The replacement of synthetic antioxidant by safer natural mixture is being suggested increasingly by the food industry nowadays. This trend has been imposed by the worldwide preference of consumers for the use of natural antioxidants, some of which may exist inherently in foods or be added intentionally during their processing. Among these, carotenoids comprise the group of the most abundant micronutrient in vegetables and fruits, and their dietary consumption is associated with lower incidence of certain types of cancer as well as with enhanced protection against cardiovascular diseases (Rai *et al.*, 2014, Kiokias and Gordon, 2004 and Agarwal, 2000). Earlier studies have indicated that the quality of the tomato is strongly correlated with its lycopene content (George *et al.*, 2004). Moreover, it is also well known that the mixture of antioxidants, with synergistic action, exert positive effect on health, associated with the consumption of fresh fruits and vegetables. Due to its high consumption rates, tomato can provide the total intake of these components significantly (Abushita *et al.*, 1997 and Beecher, 1998).

The classification between various subgenera, species and subspecies is based primarily on morphological attributes. However, these morphological characters may be unstable and influenced by environmental conditions (Goodrich *et al.*, 1985). Over the years, the methods for detecting and assessing genetic variation have extended from analysis of discrete morphological traits to biochemical and molecular traits. Genetic analysis of tomato is essential to enhance the genetic yield potential with good nutritional properties. Molecular

markers can give an effective tool for efficient selection of desired agronomic traits because they are based on plant genotypes and also independent of environment. (Franco *et al.*, 2001). Earlier studies have been reported many molecular markers viz. RFLP, AFLP, SSR, RAPD were frequently using genetic variation study in tomato crops (Hu and Quiros, 1991, Mongkolporn *et al.*, 2004, Dongre and Parkhi, 2005; Garg *et al.*, 2006 and Liu *et al.*, 2007). Random Amplified Polymorphic DNA (RAPD) is based on *in vitro* amplification of randomly selected oligonucleotide sequences. RAPD is very useful in the study of biodiversity, hybridization, gene mapping and genetic map construction (Sharma and Sharma, 1999). The aim of the present study was to evaluate and select tomato line which could be grown for good nutritional composition as well as find out the phylogenetic relationships of ten tomato lines using random amplified polymorphic DNA (RAPD) analysis.

MATERIALS AND METHODS

Ten lines of tomato (Table 1) were obtained from selected randomly selected from a replicated trail on tomato crop improvement at the Division of Vegetable Science and Floriculture, SKUAST-Jammu. Fruit sample were harvested randomly, when first fruits of the second truss reached the full ripening stage. Ten proximal fruits of each second truss were pooled from all the 3 replications, mixed thoroughly and analyzed for various biochemical parameters. Total soluble solids (TSS) were analyzed by a portable hand refractometer and the results are reported as Brix degrees at 20°C. The *pH* of tomato juice was measured using a pocket *pH* meter (HANA instruments). Titratable acidity was estimated by the method of Rangana (1976). The acidity is expressed as percent anhydrous citric acid. The Ascorbic acid content was estimated titrimetrically, using 2, 6-dichlorophenol indophenols (2, 6-DCPIP) dye, as per the method of Rangana (1976). Ascorbic acid content was calculated as ascorbic acid mg/100 g edible portion. The total carotenoids were extracted and partitioned in acetone and petroleum ether, respectively, as described by

Table 1. List of tomato lines.

No.	Genotypes	Source
1	2012/TOMATO AVT DET-1	SKUAST-Jammu
2	2012/TOMATO AVT DET-2	SKUAST-Jammu
3	2012/TOMQTO AVT DET-3	SKUAST-Jammu
4	2012/TOMQTO AVT DET-4	SKUAST-Jammu
5	2012/TOMQTO AVT DET-5	SKUAST-Jammu
6	2012/TOMQTO AVT DET-6	SKUAST-Jammu
7	2012/TOMQTO AVT DET-7	SKUAST-Jammu
8	2012/TOMQTO AVT DET-8	SKUAST-Jammu
9	2012/TOMATO Hyb DET AVT-2	SKUAST-Jammu
10	2012/TOMATO Hyb DET AVT-3	SKUAST-Jammu

Thimmaiah (1999). Absorbance measured at 452 nm and total carotenoid content (mg/100 g) was calculated using a calibration curve prepared against a high purity β carotene. Lycopene was extracted and analyzed according to Thimmaiah (1999). The absorbance was measured at 503 nm in a UV-Visible double beam Spectrophotometer (Shimadzu UV-1601). The lycopene content (mg/100 g) was calculated using molar extinction coefficient ($\Sigma = 17.2 \times 10^4$). The differences between the lines were tested using 1-way analysis of variance (ANOVA) and DMR-test was used to determine the significant differences among the test materials. Differences were considered to be significant at $P \leq 0.05$.

DNA Extraction

Genomic DNA was isolated from the young leaves of selected 10 tomato lines using CTAB method (Murry and Thompson, 1980) with few modifications. One gram of leaves was ground in liquid nitrogen to a fine powder. The powder was added to 3 ml of extraction buffer (100 mM Tris-HCl pH-8.0, 20 mM EDTA, 1.4 M NaCl, 2% CTAB and 2% β mercaptoethanol and incubated at 65°C for 30 minutes). The DNA was extracted with Chloroform: Octanol (24:1), washed with 70% ethanol and dissolved in T.E. buffer (10 mM Tris-HCl pH-8.0, 1 mM EDTA and 0.2-1 mg/ml RNase). The quality of isolated genomic DNA was checked by 0.8% agarose gel electrophoresis and quantity was estimated through mySPEC microvolume

spectrophotometer (Sigma Svi, version 1.0.0.0) nanodrop.

Molecular Analysis

Ten decamer oligonucleotide primers synthesized by IDT were used for the polymorphism survey. Amplification reactions were carried out in 25 μ L volumes, containing (10X PCR buffer, 2.5 mM dNTPs, 2.5 mM Mg Cl₂, 5 pM/ μ l primer, 3.0 μ L of genomic DNA (50 ng/ μ L 0.3 μ l), 3U/ μ L Taq polymerase. Amplifications were performed in gradient thermal cycler (Eppendorf, Germany). Programmed for an initial denaturation at 94°C for 2 min, 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 36°C and 2 min extension at 72°C followed by final extension for 10 min at 72°C.

Amplified products from the RAPD reactions were separated by horizontal gel electrophoresis unit using 2% agarose gel in TAE buffer and stained with ethidium bromide. A photographic record was taken by gel documentation system. The reproducible banding patterns of each primer which produced by RAPD were chosen for analysis. Each gel was scored as present (1) or absent (0), and pair wise comparisons between individuals were made to calculate the Jaccard's coefficient of genetic similarity matrix using NTSys software (NTSYS-pc version 2.02e). Cluster analysis was performed to produce a dendrogram using unweighted pair-group method with arithmetical

average (UPGMA).

RESULTS

Nutritional Characterization

Titrimetric analysis of ascorbic acid showed that there is significant variation in vitamin-C levels estimated in freshly harvested fruits of ten tomato lines [LSD ($P \leq 0.05$) 1.34]. In this study the vitamin-C concentration ranged from 15.82 to 31.93 mg/100 g (Table 2). The maximum ascorbic acid content was recorded in 2012/TOMATO Hyb DET AVT-3 (31.93 mg/100 g) followed by 2012/TOMATO AVT DET-1 (28.92 mg/100 g). Tomato line i.e. 2012/TOMQTO AVT DET-5, 2012/TOMQTO AVT DET-7 and 2012/TOMQTO AVT DET-8 are significantly at par. Significant variation [LSD ($P \leq 0.05$)1.34] was recorded in the total carotenoid content amongst the ten tomato lines (Table 2). The values for carotenoid ranged from

4.92 to 7.66 mg/100 g (Table 2). Maximum carotenoid content was recorded in 2012/TOMQTO AVT DET-3 (7.66 mg/100 g) followed by 2012/TOMQTO AVT DET-8 (7.04 mg/100 g). The minimum total carotenoids content were noted i.e. 4.92 mg/100 g. Significant variation in lycopene (the red pigment of tomato fruit) was also recorded [LSD ($P \leq 0.05$) 0.582] in this study and the values ranged from 3.33 to 5.68 mg/100 g. In this study, the total soluble solids (TSS) ranged between 2.50 (2012/TOMQTO AVT DET-7) and 5.43 % (2012/TOMQTO AVT DET-5) amongst the ten tomato lines (Table 3). The *pH* of tomato fruit ranged from 3.70 (2012/TOMQTO AVT DET-5) to 4.46 (2012/TOMATO Hyb DET AVT-2) amongst 10 tomato lines. The titrable acidity expressed as percentage citric acid. The acidity ranged from 0.267 to 0.560% [LSD ($P \leq 0.05$) 0.068]. The maximum acidity (0.560%) and lowest *pH* (3.70) were observed in 2012/TOMQTO AVT DET-5 (Table 3).

Table 2. Ascorbic acid, total carotenoids and lycopene content in tomato.

No.	Tomato lines	Ascorbic Acid (mg/100g)	Total carotenoids (mg/100g)	Lycopene (mg/100g)
1	2012/TOMATO AVT DET-1	28.92	4.92	3.33
2	2012/TOMATO AVT DET-2	18.53	6.31	4.38
3	2012/TOMQTO AVT DET-3	15.82	7.66	5.54
4	2012/TOMQTO AVT DET-4	20.64	6.96	5.49
5	2012/TOMQTO AVT DET-5	17.98	6.50	4.87
6	2012/TOMQTO AVT DET-6	18.30	6.45	4.27
7	2012/TOMQTO AVT DET-7	17.39	5.68	3.66
8	2012/TOMQTO AVT DET-8	17.46	7.04	5.68
9	2012/TOMATO Hyb DET AVT-2	21.21	5.14	3.43
10	2012/TOMATO Hyb DET AVT-3	31.93	6.77	5.26
	Range	15.82 -31.93	4.92-7.66	3.33- 5.68
	CD at 5%	1.34	1.23	0.862

Table 3. Variation in pH, acidity and total soluble solids (TSS) in tomato line.

No.	Tomato lines	TSS (%)	pH	Acidity (%)
1	2012/TOMATO AVT DET-1	4.66	4.36	0.343
2	2012/TOMATO AVT DET-2	4.06	4.33	0.333
3	2012/TOMQTO AVT DET-3	3.30	4.00	0.303
4	2012/TOMQTO AVT DET-4	2.56	4.00	0.483
5	2012/TOMQTO AVT DET-5	5.43	3.70	0.560
6	2012/TOMQTO AVT DET-6	4.00	4.00	0.350
7	2012/TOMQTO AVT DET-7	2.50	3.90	0.447
8	2012/TOMQTO AVT DET-8	3.36	3.96	0.387
9	2012/TOMATO Hyb DET AVT-2	3.46	4.46	0.267
10	2012/TOMATO Hyb DET AVT-3	3.80	3.83	0.493
	Range	2.50- 5.43	3.70 - 4.46	0.267 - 0.560
	CD at 5%	0.409	0.436	0.068

Molecular Characterization

Ten RAPD primers were tested against the 10 tomato lines. Out of 10, 4 primers were showed polymorphism. The sequences of these primers are listed in Table 4. The number of bands and the degree of polymorphism revealed by each primer are given in Table 4. The polymorphism percentage ranged from 50% (OPAD 05) to as high as 83.33% (OPAE 14) were noted in different primers among tomato lines. Average polymorphism across 10 tomato lines was found to be 71.53%. A total number of 35 amplified DNA bands were generated across the studied

lines with average of 8.75 bands/ primer. Out of the total band, 26 polymorphic bands were noted. Primer OPAE 14 generated maximum polymorphic bands and primer OPAD 05 produced minimum number of polymorphic bands with average 6.5 polymorphic bands per primer. The polymorphism Information content (PIC) ranged from 0.759-0.385 with average of 0.612. The highest PIC was estimated with primer OPAE 14 (0.759) whereas primer OPAD 05 showed least PIC value (Table 4).

The average genetic similarity among the 10 tomato lines was 0.63 with a range of 0.33-0.93 (Table 5).

Table 4. List of primer and sequence, polymorphism (%) and number of bands.

No.	Primer	Sequence	GC content (%)	Polymorphism (%)	Total no. of bands	Polymorphic band	PIC
1	OPAE 11	5'AAGACCGGA3'	60%	77.77%	9	7	0.713
2	OPAD 05	5'ACCGCATGGG3'	70%	50.00%	6	3	0.385
3	OPAE 14	5'GAGAGGCTCC3'	70%	83.33%	12	10	0.759
4	OPAE 09	5'TGCCACGAGG3'	70%	75.00%	8	6	0.612

PIC - Polymorphic information content

Table 5. Similarity coefficient among tomato lines induced by RAPD primers.

	2012/TOMATO AVT DET-1	2012/TOMATO AVT DET-2	2012/TOMQTO AVT DET-3	2012/TOMQTO AVT DET-4	2012/TOMQTO AVT DET-5	2012/TOMQTO AVT DET-6	2012/TOMQTO AVT DET-7	2012/TOMQTO AVT DET-8	2012/TOMATO Hyb DET AVT-2	2012/TOMATO Hyb DET AVT-3
	1	2	3	4	5	6	7	8	9	10
1	1.00									
2	0.63	1.00								
3	0.83	0.53	1.00							
4	0.52	0.63	0.50	1.00						
5	0.67	0.52	0.77	0.57	1.00					
6	0.63	0.57	0.71	0.62	0.93	1.00				
7	0.43	0.54	0.48	0.52	0.42	0.46	1.00			
8	0.46	0.50	0.38	0.48	0.34	0.33	0.74	1.00		
9	0.63	0.50	0.71	0.48	0.59	0.56	0.67	0.54	1.00	
10	0.46	0.50	0.38	0.59	0.44	0.43	0.62	0.73	0.54	1.00

The average genetic similarity among the 10 tomato lines was 0.63 with a range of 0.33-0.93 (Table 5). The highest similarity value was 0.93 which recorded between 2012/TOMQTO AVT DET-5 and 2012/TOMQTO AVT DET-6, while the lowest similarity value i.e. 0.33 was observed between 2012/TOMQTO AVT DET-6 and 2012/TOMATO AVT DET-8 (Table 5). The cluster analysis was performed to further elucidate the relationship among the tomato lines. Similarity coefficient matrices were used to generate a dendrogram of tomato genotypes based on UPGMA analysis (Figures 1 and 2), the analysis divided 10 tomato lines into 3 distinct clusters *i.e.*, Cluster I, II, and III. The

cluster I comprised 5 tomato lines *i.e.*, 2012/TOMATO AVT DET-1, 2012/TOMQTO AVT DET-5, 2012/TOMQTO AVT DET-3, 2012/TOMQTO AVT DET-6 and 2012/TOMATO Hyb DET AVT-2. The highest similarity value of 0.93 were recorded between 2012/TOMQTO AVT DET-5 and 2012/TOMQTO AVT DET-6. The Cluster II comprised only 2 tomato lines *i.e.*, 2012/TOMQTO AVT DET-2 and 2012/TOMQTO AVT DET-4 with similarity coefficient of 0.63. A total of 3 tomato lines were grouped in cluster III *i.e.*, 2012/TOMQTO AVT DET-7, 2012/TOMQTO AVT DET-8 and 2012/TOMATO Hyb DET AVT-3.

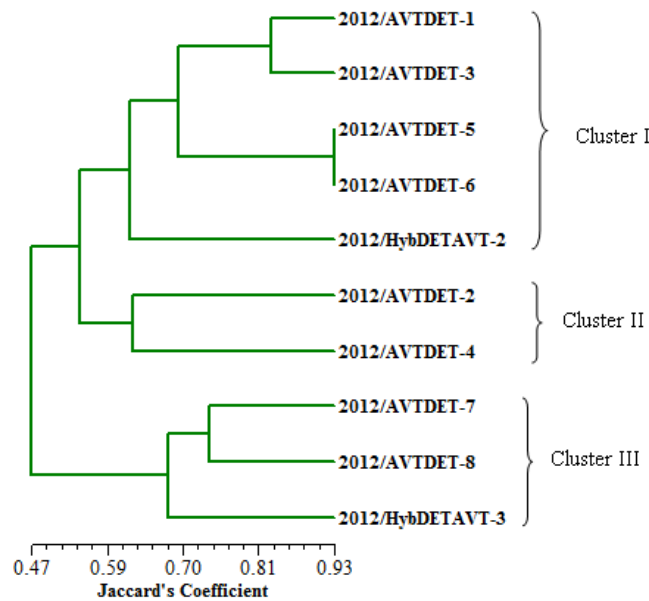


Figure 1. Dendrogram of 10 tomato lines produced by UPGMA clustering method based on the genetic similarity (Tomato line name are given as per serial number in Table 1).

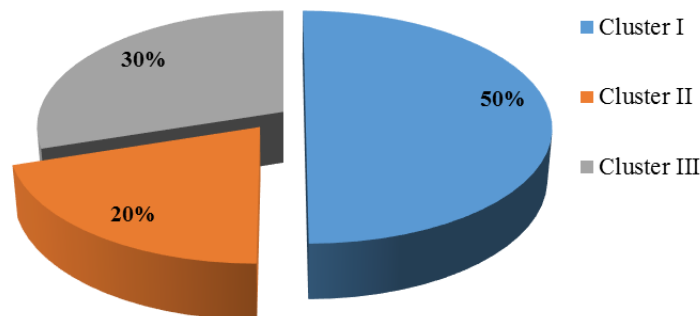


Figure 2. Grouping of tomato lines based on similarities.

Principal components analysis (PCA) was used to identify multidimensional relationships that describe portions of the genetic variance in a data set (Figure 3). Ten tomato lines were used in order to elucidate their genetic diversity by using molecular markers. On the molecular level, 4 primers were used to differentiate

between these varieties and gave reproducible results with wide variations in their band numbers. The molecular markers obtained by the RAPD technique revealed a remarkable molecular discrimination between the ten tomato varieties under the study.

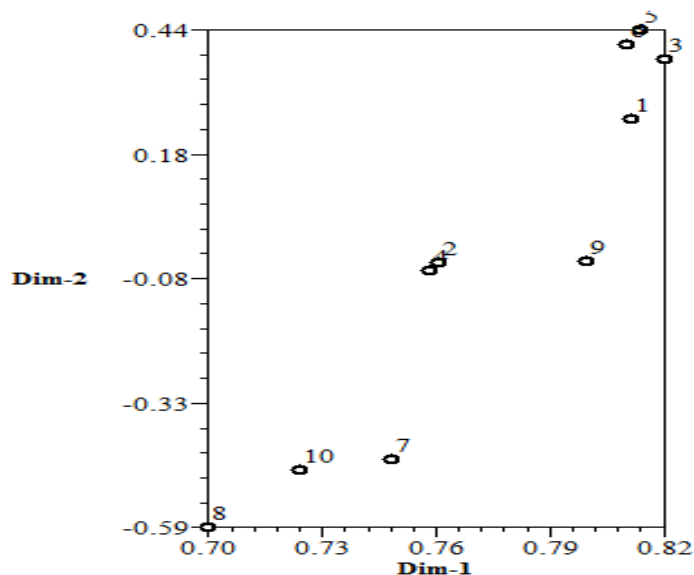


Figure 3. Principal component graph of 10 tomato line derived from RAPD (Tomato line number corresponds to serial number shown in Table 1).

DISCUSSION

Identification of tomato lines with higher nutritional value is advantageous for crop improvement. The large variation in vitamin C level has been noted among tomato lines. Similar findings were reported by Rai *et al.*, (2014). Singh *et al.*, (2004) was reported that ascorbic acid content ranged from 11.21 to 53.29 mg/100 g in 15 cultivars of tomato. Sharma *et al.*, (1996) reported ascorbic acid content ranged from 11.21 to 53.29 mg/100 g in 53 genotypes of tomato. The biological function of vitamin C is based on its ability to donate electrons, which provides intra- and extra-cellular reducing power for a variety of biochemical reactions. In mammalian cells, vitamin-C serves as a co-factor for reactions that require reduced iron and or copper metallo-enzymes (Tsao, 1997). Substantially high cellular levels of vitamin-C provide antioxidant protection against

photosynthetically generated free radicals (Delamere, 1996). Another important indirect function of vitamin C is its ability to regenerate other biologically important antioxidants such as glutathione and vitamin E into their reduced state (Jacob, 1995). The vitamin A activity of tomato fruit is determined mainly by the carotenoids content, thus the tomato cultivars were also evaluated for total carotenoids. The total carotenoids content values recorded in this study confirms those reported by Singh *et al.*, (2007) who reported that the total carotenoids values varied from 1.00 to 9.47 mg/100 g in 40 tomato genotypes. Raffo *et al.*, (2002) reported that the carotenoids content of tomato were very low at the breaker stage (1.08 mg/100 g), which increased ≥ 10 -fold during ripening and reached 12.705 mg/100 g at full ripening stage. In earlier studies, Rai *et al.*, (2012) showed similar finding in Indian tomato genotypes. The values of lycopene are in close proximity to the published

data on different varieties from India (Singh *et al.*, 2007; Rai *et al.*, 2012 and 2014) and to those of Clinton (1998) who reported that the yellow cultivars contain about 0.5 mg/100 g and the red ones as high as 9.0 mg/100 g. Audrius *et al.*, (2009) reported that the lycopene content in luthiana tomato varied from 8.55-13.56 mg/100 g. Abushita *et al.*, (1997) reported that the lycopene content in 12 tomato cultivars, which ranged from 5.180 to 8.470 mg/100 g. Lycopene is the most abundant carotene in red tomato fruits, accounting for 90% of the total amount of carotenoids (Audrius *et al.*, 2009). Typical red pigmented tomato fruits also contain lesser amount of β carotene and other carotenoids. Other quality parameters, viz. *pH*, acidity and total soluble solids (TSS), essential for flavor and processing needs, were also estimated. The total soluble solids are composed of all fruit components except water and those volatilized during drying. About 50% of the dry matter is composed of sugars, primarily reducing sugars, glucose and fructose and the quantity of sucrose is negligible. Also, minute quantities of saccharose, raffinose, arabinose, xylose, galactose and sugar alcohol mynositol have been reported.

Acids not only contribute to sourness of tomato fruits but also are major factor in flavor intensity (Stevens *et al.*, 1979). Organic acids comprise about 15% of dry content of fresh tomatoes. Citric and mallic acids are the major organic acids, in addition to several other carboxylic acids, sugars acids and alicyclic acids. Citric acid is usually the predominant acid in tomato fruits and it usually constitutes about 40-90% of the organic acids. In the ripe red tomato, mallic to citric acid ratio is 0.5 or lower. Malic acid has been reported to be 14% more sour than citric acid, but it has less influence on tomato taste because of its lower concentration. TSS, *pH* and acidity values recorded in this study confirm those reported by Rai *et al.*, (2012) found that the *pH* ranged from 3.71-4.37 and acidity ranged from 0.36-0.57. Singh *et al.*, (2007) who reported that TSS ranged from 3.06-6.13%, *pH* varied from 3.76 to 4.56, and acidity (citric acid) range from 0.202 to 0.710% amongst 40 genotypes of tomato. Stevens *et al.*, (1977) showed that fructose and citric acid were more important to sweetness and sourness,

rather than glucose and malic acid and *pH* was a better objective measure of sourness than titratable acidity. It has shown that a high acid and a higher sugar concentration in tomato fruit generally improve the organoleptic quality and flavour in tomato.

Molecular characterization was carried out through RAPD molecular technique by using 10 decamer primers, out of which four primers showed polymorphism. The 4 primers generated 35 loci in all tomato lines. Maximum number of loci (12) was noted in genome of OPAE 14 and minimum number of loci (6) in the genome of OPAE 05. Polymorphism was estimated between 10 tomato lines by 10 decamer primers with different sequence out of which 4 primers showed about 71.53% polymorphism, in all tomato lines. By using eight decamer RAPD primers, 228 loci were found among 36 tomato cultivars (Huh *et al.*, 2011). Seventy four amplified bands were scored with 62.2% of polymorphism in 14 tomato genotypes were reported by Ezekiel *et al.*, (2011). The application of both biochemical and molecular genetics techniques have an important potential to provide a new tool for the study of both wild and domesticated species in respect to investigation of evolution and migration of species from their gene pool centers (Fregonezi *et al.*, 2006). The identification and characterization of species become possible through fingerprinting for each species since DNA is a source of informative polymorphism (El-Rabey, 2008), consequently, techniques of molecular genetic markers have an important potential for the detection of genetic differences among species (Benmoussa and Achouch, 2005). Munazza *et al.*, (2009) reported that the assessment of genetic diversity within and between landraces should have priority for varieties improvement. At the same time it is necessary to develop better methods of characterization and evaluation of germplasm collections, to improve strategies for conservation and collection of germplasm and to increase the utilization of plant genetic resources. Phylogenetic dendrogram was constructed among selected varieties using RAPD fingerprints through computerized software. Elhaman *et al.*, (2010) and Ezekiel *et al.*, (2011) studied the genetic diversity in

tomato using RAPD-PCR technique. Thus tomato is an excellent source of nutrients, especially vitamin C, total carotenoids as well as lycopene, which are the major contributors to the antioxidant activity of the fruit. The maximum ascorbic acid content was recorded in 2012/TOMATO Hyb DET AVT-3 followed by 2012/TOMATO AVT DET-1 whereas the maximum total carotenoids content was recorded in 2012/TOMQTO AVT DET-3, 2012/TOMQTO AVT DET-8 and 2012/TOMQTO AVT DET-4. The maximum lycopene was recorded in 2012/TOMQTO AVT DET-8, 2012/TOMQTO AVT DET-3 and 2012/TOMQTO AVT DET-4.

The information related to the significant variability of these antioxidant phytochemicals in the tomato observed in this study can be utilized in the breeding programme to develop tomato genotypes with higher antioxidant potential. It is concluded that RAPD marker are effective in assessing and discriminating the tomato lines. Therefore, the use of RAPD markers in the applied breeding programmes can facilitate appropriate choice of parents involved for crosses.

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REFERENCES

- Abushita AA, Daood HG and Biacs PS (2000). Changes of carotenoid and antioxidant vitamins in tomato as a function of varietal and technological factors. *Journal of Agricultural and Food Chemistry* 48 (2): 75-81.
- Abushita AA, Hebshi EA, Daood HG and Biacs PS (1997). Determination of antioxidant vitamins in tomato. *Food chemistry* 60: 207-212.
- Agarwal S and Rao AV (2000). Tomato lycopene and its role in human health and chronic disease. *Canadian Medical Association Journal* 163: 739-744.
- Audrius R, Rasa K, Ceslovas B and Pranas V (2009). Nutrition quality of different tomato cultivars. *Zemdirbyste-Agriculture* 96 (3): 67-75.
- Beecher GR (1998). Nutrient content of tomatoes and tomato products. *Proceeding of Society of Experimental Biology and Medicine* 218: 98-100.
- Benmoussa M and Achouch A (2005). Effect of water stress on yield and its components of some cereals in Algeria. *Journal of Central European Agriculture* 6 (4): 427- 434.
- Beutner S, Bloedorn B, Frixel S, Blanco IH, Hoffman T, Martin H (2001). Quantitative assessment of antioxidant properties of natural colorants and phytochemicals: carotenoids, flavonoids, phenols and indigoids. The role of β -carotene in antioxidant functions. *J. Sci Food Agric.* 81:559–568.
- Buta JG and Spaulding DW (1997). Endogenous level of phenolics in tomato fruits during growth and maturation. *Journal of Plant Growth Regulator* 16: 43-46.
- Clinton S (1998). Lycopene: Chemistry biology and implication for human health and disease. *Nutritional Review* 56: 35-51.
- Delamere NA (1996). Ascorbic acid and the eye subcell. *Biochemistry* 25: 313-329.
- Dragan Z and Tomaz P (2006). Comparative study of quality changes in tomato cv. 'Malike' (*Lycopersicon esculentum* Mill.) whilst stored at different temperatures. *Acta agriculturae Slovenica* 87 (2): 235-243.
- Dumas Y, Dadomo M, Di Lucca G and Grolier P, (2003). Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *J. Sci Food Agric.* 83:369–382.
- Elham AA, Hady AE, Atef AA, Haiba S, Nagwa R, Hamid AE and Aida A (2010). Phylogenetic diversity and relationships of some tomato varieties by electrophoretic protein and RAPD analysis. *J. American Sci.*, 6 (11): 434-441.
- El-Rabey H (2008). Molecular and biochemical studies on Egyptian *Hordium murinum* L. complex as revealed by RAPD-PCR and seed storage protein electrophoresis. *Taeckholmia* 28: 145-156.
- Ezekiel CN, Nwangburuka C, Ajibade OA and Odebode AC (2011). Genetic diversity in 14 tomato (*Lycopersicon esculentum* Mill.) varieties in Nigerian markets by RAPD-PCR technique. *African J. Biotech.* 10 (25): 4961-4967.

- FAO, *Crop Description and Climate*, (2004). Available: <http://www.fao.org/ag/agl/aglw/cropwater/tomato.stm#-descrip> [24 January 2005].
- Fregonezi JN, Fernandes T, Domingues T, Vieira A and Vanzela A L (2006). Karyotype differentiation of four *Cestrum* species (*Solanaceae*) based on the physical mapping of repetitive DNA. *Genetics and Molecular biology* 29 (1): 97-104.
- Gahler S, Otto K and Bohm V (2003). Alterations of vitamin C, total phenolics and antioxidant capacity as affected by processing tomatoes to different products. *J. Agric. Food Chem.* 51:7962-7968.
- George B, Kaur C, Khurdiya DS and Kapoor HC (2004). Antioxidants in tomato (*Lycopersicon esculentum* L.) as a function of genotype. *Food Chem.* 84:45-51.
- Huh M.K, Youn SJ and Kang SC (2011). Identification and genetic diversity of Korean tomato cultivars by RAPD markers. *J. Life Sci.* 21 (1): 15-21.
- Jacob RA (1995). The integral antioxidant system. *Nutritional Research* 15: 755-766.
- Kiokias S and Gordon M (2004). Antioxidant properties of carotenoids *in vitro* and *in vivo*. *Food Review International* 20: 99-121.
- Munazza S, Salman AM, Malik AR and Pearce SR (2009). Electrophoretic characterization and the relationship between some Brassica species. *Electronic Journal of Biology* 5 (1): 1-4.
- Murray MG and Thompson WF (1980). Rapid isolation of high molecular weight plant DNA. *Nuclie Acid Research* 8 (19): 4321-4326.
- Raffo A, Leonari C, Fogliano V, Ambrosino P, Salucci M, Gennaro L, Bugianesi R, Giuffrida F and Qualgia G (2002). Nutritional value of cherry tomatoes (*Lycopersicon esculentum* Cv. Naomi F1) harvested at different ripening stages. *Journal of Agriculture and Food Chemistry* 50: 6550-6556.
- Rai Gyanendra K, Kumar R, Kumar RR and Dogra S (2014). Free radicals scavenging - antioxidant phytochemicals in cherry tomato (*Solanum Lycopersicon* var. *Ceresiforme* (dunal) a. Gray). *Bangladesh J. Bot.* 43 (3): 255-260.
- Rai Gyanendra K, Kumar R, Singh AK, Rai M and Chaturvedi AK (2012). Changes in antioxidant and phytochemical properties of tomato (*Solanum lycopersicum* L.) under ambient condition. *Pak. J. Bot.* 44 (2): 667-670.
- Ranganna S (1976). Handbook of analysis and quality control for fruits and vegetable products. Tata Mc Graw Hill Publishing Co. Ltd. New Delhi. Edition 2, pp 545.
- Rao AV, Waseen Z and Agarwal S (1998). Lycopene content of tomatoes and tomato products and their contribution to dietary lycopene. *Food Res Int.* 31: 737-741.
- Rao AV and Agarwal S (2000). Role of antioxidant lycopene in cancer and heart disease. *J Am Coll Nutr* 19: 563-569.
- Sharma S, Mahajan R and Bajaj KL (1996). Biochemical evaluation of some tomato varieties. *Vegetable Science* 23(1): 42-47.
- Singh J, Rai M, Kumar R, Verma A and Rai GK (2007). Genotyping variation and hierarchical clustering of tomato (*Lycopersicon esculentum* Mill.) based on morphological and biochemical traits. *Vegetable Science* 34(1): 40-45.
- Singh J, Rai GK, Upadhyay AK, Kumar R and Singh KP (2004). Antioxidant phytochemicals in tomato (*Lycopersicon esculentum* Mill.). *Indian Journal of Agricultural Sciences* 74 (1): 3-5.
- Stevens MA, Kader AA, Albright-Holton M and Algazi M (1977). Genotype variation for flavor and composition in fresh market tomato. *Journal of American Society of Horticultural Sciences* 102: 680-689.
- Thimmaiah SK (1999). Standard method of Biochemical analysis, Kalyani Publisher New Delhi.
- Toor RK and Savage GP (2005). Antioxidant activity in different fractions of tomatoes. *Food Res Int.* 38: 487-494.
- Tsao CS (1997). An overview of ascorbic acid chemistry and biochemistry. In: L. Packer, and J. Fuch, eds., *Vitamin C in health and diseases*. Marcal Dekker, New York.