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Internal quality characterization and isolation of lycopene specific genes from tomato

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Abstract

Tomato (*L. esculentum* Mill), a popular vegetable in tropics is an excellent source for vitamin A, C, carotenoids and other health related components. It tops the list of industrial crops because of its outstanding processing qualities. It is valued for both its fresh and processed forms. Biochemical analysis in different wild species, varieties and hybrids of tomato showed the wild species, *Lycopersicon pimpinellifolium* LA 1593 to be a rich source for lycopene specific genes. Partial cDNA of lycopene specific *Phytoene desaturase* gene TNAU P was isolated from *L. pimpinellifolium* LA 1593 by RT-PCR technique. Sequence analysis of the partial cDNA showed 99.6% similarity with already available *Phytoene desaturase* gene from *L. esculentum*. Also, the sequence showed considerable homology with *Phytoene dehydrogenase*, *Zeta carotene desaturase* and *Phytoene desaturase* genes from Gentian, *Oryza, Momardica,* citrus and pea. The high intensity of the amplified product in *L. pimpinellifolium* coupled with 99.6 % homology to *L. esculentum* inferred that the level of expression of *Phytoene desaturase* is more in *L. pimpinellifolium*. Isolation of *Phytoene desaturase* genes can be further exploited to produce transgenic plants with increased content of lycopene by transferring the genes from wild species to cultivars.

Key words: *Lycopersicon pimpinellifolium, Phytoene desaturase,* RT-PCR, lycopene

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops grown throughout the world for fresh consumption as well as for processing. In terms of human health, tomato is a major component of daily meals in many countries and constitutes an important source of minerals, vitamins and antioxidant compounds. Tomato fruit quality for fresh consumption is determined by appearance (color, size, shape), firmness, texture, dry matter, organoleptic (flavor) and nutraceutical (health benefit) properties. The organoleptic quality of tomato is mainly attributed to its aroma volatiles, sugar and acid content, while the nutraceutical quality is defined by its mineral, vitamin, carotenoid and flavonoid content.

Several works have established the role of soluble solid content (SSC), acids and sugars in the taste and flavour intensity of tomato fruits. It is well known that sweetness has a high correlation with SSC, pH and reducing sugars, and sourness has a high correlation with pH and, to a lesser degree with titrable acidity (TA) (Baldwin *et al.*, 1998). This happens because the major constituents of SSC content in *Lycopersicon* fruits are all soluble sugars. The pH and TA are good measures of free acid and H-ion concentrations, responsible for the sourness of a solution (Stevans *et al.*, 1977). Moreover SSC, sugars and TA highly contribute to overall flavour intensity (Baldwin *et al.*, 1998). Nevertheless, SSC, pH and TA are ambiguous variables since the profile and content of the substances that contribute to them can vary greatly between accessions. These variations can be large if accessions of different species related to cultivated and wild tomato are characterized.

The most abundant phytonutrients in tomatoes are the carotenoids (Gann *et al.*, 1999). Lycopene is the most prominent carotenoid followed by gamma carotene, zeta-carotene, phytofluene, phytoene, neurosporene and lutein (Leonardi *et al.*, 2000; Clinton, 1998). Research indicates that lycopene supplements and lycopene-rich foods such as tomato sauce support fertility, promote heart health, protect against Alzheimer's disease and several types of cancer (Hadley *et al.*, 2002). Tomato also contain other components that are beneficial to human health including vitamin A, trace elements, flavonoids, phytosterols, several water soluble vitamins and two other cancer-fighting phytochemicals, courmaric and chlorogenic acids (Campbell *et al.*, 2004).

Although tomato is a source of phytonutrients, the levels are not sufficient to meet out the daily requirements. Also, compared to wild varieties of tomato, current tomato cultivars are relatively low in the important phytochemical, lycopene. Among wild species of tomato *L. pimpinellifolium* is reported to be a potential source of genes for specific antioxidants like lycopene, total phenolics and beta carotene. *L. pimpinellifolium,* also known as the currant tomato, produces tiny fruits which contain over 40 times more lycopene than domesticated tomatoes (Grolier and Rock, 1998).

The objective of this work is to characterize and classify different tomato genotypes according to their usefulness for internal quality breeding programs of fresh tomato. The variables considered in this study are related to the nutritional and qualitative aspects, emphasizing the sugar, acid composition and carotenoids of the samples. Also isolation and characterization of lycopene specific genes was carried out from a wild species *L. pimpinellifolium.*

Materials and methods

Plant materials: The plant materials used in the present study includes young fruits of *Lycopersicon spp*. Wild species used in the study included *L. peruvianum* - EC 52071 obtained from NBPGR, New Delhi and *L. pimpinellifolium* – AC No LA 1593, LA 1582, LA 1586 obtained from AVRDC, Taiwan. Other local cultivars used in the study included CO 3, PKM 1 and hybrids, Ruchi, COLCRH 1, COLCRH 2, COLCRH 3 collected from Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore.

Analytical methods: Fresh fruits were homogenized in a laboratory blender. Aliquots were taken to analyse pH, total soluble solids, titrable acidity, vitamin C, total sugars, carotenoids and lycopene. pH was determined by using ELICO LI 127 pH meter. Total soluble solids content was determined by using a 'Zeiss' hand refractometer and the results are reported as ^{o}Brix. Total sugars and reducing sugars were quantified spectrophotometrically of soluble sugars and available carbohydrates, after hydrolysis of complex carbohydrates with hydrochloric acid using Nelson's arseno molybdic reagent (Somogyi, 1952). Titrable acidity was measured by titration with 0.1N NaOH and phenolphthalein, and the results were expressed as percent citric acid (A.O.A.C, 1975). Ascorbic acid was measured by titration with 4% oxalic acid and 2,6, dichlorophenol indophenol dye solution and the results were expressed as mg/100g (A.O.A.C, 1975). Total carotenoids and lycopene were quantified spectrophotometrically and expressed as mg/100 g of sample (Ranganna, 1979).

Statistical analysis: An analysis of variance (ANOVA) was performed on the data for different genotypes related to quality parameters, followed by mean separation with Fisher's protected least significant difference test (PLSD, at P=0.05). All the statistical work were analyzed using AGRES Version 7.01 software to determine differences between means.

Gene isolation studies: For RNA isolation, the frozen pericarp tissues of different wild species/ cultivars/ hybrid tomato genotypes were ground to a fine powder in liquid nitrogen, homogenized and total RNA was isolated as described by Chomczynski and Sacchi (1987) using TRIzol reagent. mRNA was isolated separately from total fruit RNA using GenElute™ mRNA Miniprep Kit (Sigma Aldrich Chemicals Pvt. Ltd., USA).

For cloning the cDNA and determining the expression levels, reverse transcription of the isolated fruit mRNA was performed in a 20µl reaction mixture containing: 50 ng of mRNA, RNAse inhibitor 1.0 µL, 0.1 M DTT 1.0 µl, RT buffer (5X) 4.0 µL, 30 mM dNTP mix 2.0 µL, AMV reverse transcriptase 0.5 µL (Bangalore Genei). The samples were incubated at 42°C for 1 h and thereafter the reverse transcriptase was inactivated by heating at 97°C for 5 min.

Gene specific primers were designed based on the already available sequence for *Phytoene desaturase* gene from *L. esculentum* (Acc. No. X59948). The primer sequences were as follows:

TNAU P F : 5' TGGAGGCAAGGGATGTTC 3'
TNAU P R : 5' GCTTCACCTCGCACTCTTCTTC 3 '
PCR reactions were performed in a total volume of 50µL containing: First strand cDNA 3.0 µL, 10X PCR buffer 5.0 µL, 30 mM dNTP mix (7.5 mM each) 1.0µL, forward gene specific primer (10 pmol/µL) 1.5 µL, reverse gene specific primer (10 pmol/µl) 1.5 µL, *Taq* DNA polymerase (3 U/µL) (Genei) 1.0 µL, sterile water 37.0 µL in 0.2mL thin walled tubes, using thermal cycler (Epppendorf). The PCR cycling profile was denaturation at 92°C for 1 min, annealing at 60°C for 1 min and an extension at 72°C for 1.5 min for 30 cycles. The amplification products were separated by electrophoresis on 1% agarose gel containing ethidium bromide and photographed using Alpha Imager TM 1220 Documentation and Analysis Systems. The amplified cDNA products obtained in PCR reactions were loaded separately on 1.5% low melting agarose gel, electrophoresed and eluted using GenElute™ Gel Extraction Kit (Sigma Aldrich Chemicals Pvt. Ltd., USA).

Cloning and sequencing of TNAU P cDNA: The eluted 1400 bp cDNA product obtained from RT-PCR using the above gene-specific primers were cloned in pTZ57R/T vector (InsT/A clone™ PCR Product Cloning Kit, MBI Fermentas Inc., USA) using T/A cloning strategy. Sequencing of the clone was done using the M13 primer sequences present in the pTZ57R/T vector in a automated sequencer model 3100 version 3.0 (ABI PRISM) at the DBT- supported DNA sequencing facility at University of Delhi South Campus, New Delhi.

Analysis of the sequence data of the cDNA clone was performed using various bioinformatics tools *viz.*, BLAST, BLASTp, CLUSTALW, FASTA, GeneRunner and Treetop.

Table 1. Quality parameters in different wild species/varieties/hybrid tomato

Sl. No.	Species	pH	Total soluble solids (°Brix)	Titrable acidity (%)	Ascorbic acid (mg 100g^{-1})	Total sugars (mg 100g^{-1})	Reducing sugars (mg 100g^{-1})	Carotenoids (mg 100g^{-1})	Lycopene (mg 100g^{-1})
1.	COLCRH 1	4.18	4.7	0.51	23.38	4.14	3.96	3.91	6.39
2.	COLCRH 2	4.29	4.69	0.52	23.29	4.18	3.43	3.72	6.84
3.	COLCRH 3	4.28	4.65	0.54	22.68	4.16	3.20	3.94	6.61
4.	Ruchi	4.18	4.9	0.52	23.84	4.22	3.06	3.52	6.54
5.	CO 3	4.16	4.74	0.51	23.29	4.32	4.29	3.78	6.42
6.	PKM 1	4.26	4.70	0.54	23.74	4.07	3.34	3.70	6.53
7.	*L. pimpinellifolium* LA 1593	5.26	5.32	0.30	25.48	3.69	3.87	4.47	7.12
8.	*L. pimpinellifolium* LA 1582	5.28	5.33	0.32	25.42	3.44	3.62	4.36	7.05
9.	*L. pimpinellifolium* LA 1586	5.22	5.36	0.33	24.80	3.45	3.75	4.42	7.07
10.	*L. peruvianum* EC 52071	5.38	5.28	0.34	25.97	3.86	3.79	4.54	6.85
Grand Mean		4.65	4.97	0.44	24.19	3.93	3.63	4.04	6.74
SEd		0.03	0.02	0.02	0.02	0.10	0.03	0.03	0.03
CD (P=0.05)		0.06	0.03	0.04	0.05	0.02	0.06	0.08	0.06

Results and discussion

Fruits of different wild species, varieties and hybrids were utilized for estimating different biochemical parameters and the results of the analysis are shown in Table 1.

The pH and titrable acidity (TA) are good measures of free H^+ ion concentration responsible for the sourness of a solution (Stevans *et al.*, 1977). A great pH variability was observed (4.16-5.38) and the results clearly separate *L. peruvianum* from *L. pimpinellifolium* accessions. Lower pH values were observed for cultivar CO 3 (4.16). Hybrids selected in this study showed pH values in the range of 4.18 - 4.29. Thangam (1998) reported pH values of 4.18 and 4.29 for hybrids Iswaraya Lakshmi and Sadabahar, respectively. However the wild species, *L. peruvianum* and *L. pimpinellifolium* species showed a higher pH range (5.22-5.38). Based on the pH values, the wild species, varieties and hybrids can be classified for their acidic nature. All the varieties and hybrids were grouped under 'acid I' category (pH 4.6-3.7), as they had pH values between 4.3-4.2. The wild species *L. pimpinellifolium* was grouped under 'semi acid' (pH 5.3-4.6) as all the accessions studied had pH values in the range of 5.3-5.2. *L. peruvinaum* was grouped under 'non-acidic' pH 7.0-5.3 as it had a pH value of 5.4.

The total soluble solids (TSS) is an important trait for processing tomato. The flavour of the tomato products depends upon the TSS content of the fruits (Ruiz *et al.*, 2004). The TSS of different varieties and hybrids chosen for this study ranged from 4.65- 4.90 °Brix. Raghupathy (2004) reported a TSS of 4.7 °Brix in PKM 1. Thangam (1998) reported a TSS of 4.65 °Brix in the hybrid, IHR-709 and 4.90 in the hybrid, Avinash. Higher mean value of 5.36 °Brix were observed in the small fruited wild species *L. pimpinillifolium* LA 1586. Other wild species chosen in the study recorded a TSS in the range of 5.28 - 5.33 °Brix.

The nutritive quality of the tomato fruit is also assessed by the acidity and ascorbic acid content. A hybrid with low acid content is preferred for fresh market. But for processing purpose, varieties with high acid content (0.50 %) is desirable. In the present study all varieties and hybrids gave a titrable acidity greater than 0.50 %. Thamarai Selvan (2004) reported acidity values of 0.5 and 0.54 % for CO 3 and PKM 1, respectively.

Minimum level of ascorbic acid needed for processing is 23mg $100g^{-1}$ and for canning purposes is 17mg $100g^{-1}$. The varieties and hybrid chosen in the study recorded ascorbic acid contents in the range of 24–26 mg $100g^{-1}$ indicating their suitability for processing purposes.

Apart from nutritional benefits, ascorbic acid is reported to have a role in nematode resistance. The role of ascorbic acid, ascorbate oxidase, hydroxyproline containing proteins imparting root-knot resistance has been reviewed by Arrigoni (1979). It has been reported that increase in the concentration of ascorbic acid leads to enhanced synthesis of hydroxyl proline rich proteins which in turn is reflected as resistance expression. In the present study, *L. peruvianum* had the highest ascorbic acid content of about 25.97 mg $100g^{-1}$. Also *L. peruvianum* was found to be resistant to root knot nematode. The increased ascorbic acid content of *L. peruvianum* may attribute to its increased nematode resistance.

The sugars make an important contribution to the flavour of

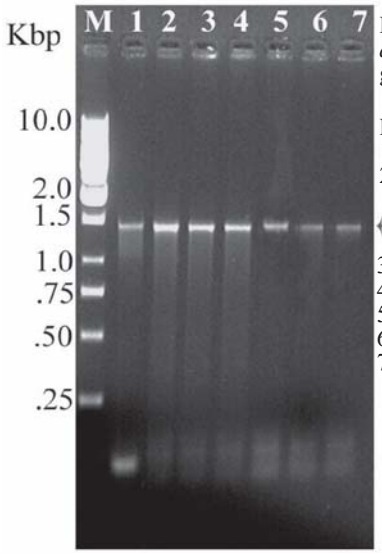

Fig. 1. Amplified *Phytoene desaturase* cDNA in different genotypes of tomato

M- 1 Kb DNA ladder,
1- *L. peruvianum* EC 52071,
2- *L. pimpinellifolium* LA 1593,
3- *L. pimpinellifolium* LA 1586,
4- *L. pimpinellifolium* LA 1582,
5- CO3,
6- PKM 1,
7- Ruchi

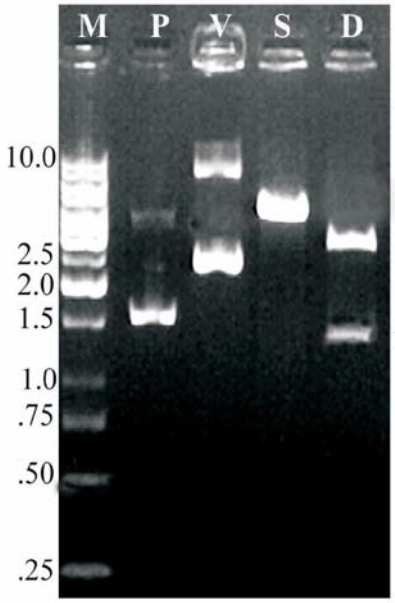

Fig. 2(a). Confirmation of recombinant clones by restriction digestion

M- 1 Kb DNA ladder
P- Undigested plasmid with insert
V- pTZ 57R/T vector
S- Restricted plasmid digested with *Xba* I
D- Restricted plasmid digested with *Xba* I and *BamH* I
Arrows indicate released cDNA fragments after digestion

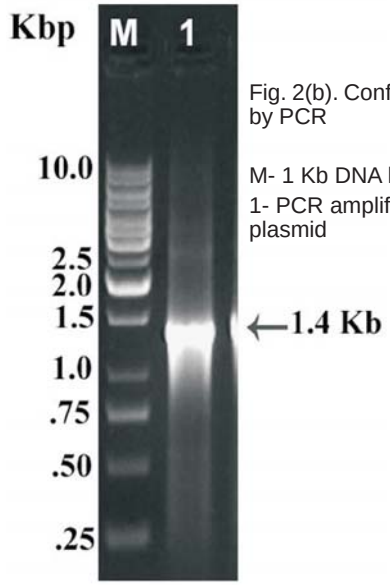

Fig. 2(b). Confirmation of recombinant clones by PCR

M- 1 Kb DNA ladder
1- PCR amplified products from recombinant plasmid

tomato and generally the reducing sugars contribute at least to 50% of total sugars. In the present study, a total sugar content of 4.32% and a reducing sugar content of 3.96% were observed in cultivar CO 3. High total sugar of 3.94% was reported by Khandaker *et al.* (1994) in a tomato variety, Pelican. There was not much difference between wild species and other cultivars with respect to total and reducing sugar contents.

Bright red colour of the fruit is one of the quality factors which is due to lycopene content. It is an important attribute for fresh market as well as for processing (Nicolle *et al.*, 2004; Fraser *et al.*, 1994; Ronen *et al.*, 2000). In the present study, the wild species, *L. pimpinellifolium* LA 1593 had the highest lycopene content (7.12 mg) among all the samples studied. Besides imparting colour, lycopene is also reported to be an antioxidant which protects against several types of cancer (Hadley *et al.*, 2002; Canene-Adams *et al.*, 2005).

Using first strand cDNA obtained from fruit mRNA of wild species, variety and hybrid fruits as template and with phytoene desaturase specific forward (5') and a reverse (3') primers the second strand cDNA synthesis was carried out. A cDNA fragment of 1.4 Kb were obtained in all the samples analyzed (Fig. 1). The cDNA fragments were intense in all the *L. pimpinellifolium* accessions when compared to the varieties CO 3, PKM 1 and hybrid Ruchi lanes and the wild species *L. peruvianum* EC 52071. The cDNA fragment of 1.4 Kb obtained by RT-PCR using the gene specific primers from *L. pimpinellifolium* LA 1593 was cloned in pTZ57R vector (Invitrogen) using T/A cloning strategy. The recombinant cDNA clone was confirmed for the presence of insert using restriction

Fig. 3. Multiple sequence alignment of TNAU P with other carotenoid genes

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TNAU P                          ------------SRMHLDLEARDVLGGKVAAWKDDDGDWYETGLHIVFGAYPNIQNLFG 47
PHYTOENE DEHYDROGENASE          LGGLSTAKYLADAGHKPILLEARDVLGGKVAAWKDDDGDWYETGLHIFFGAYPNIQNLFG 180
GENTIAN PDS                     LAGLTTAKYLADAGHKPILLEARDVLGGKVAAWKDDDGDWYETGLHIFFGAYPNVQNLFG 176
CITRUS PDS                      LAGLSTAKYLADAGHKPLLLEARDVLGGKVAAWKDGDGNWYETGLHIFFGAYPNIQNLFG 149
ORYZA PDS                       LAGLSTAKYLADAGHKPILLEARDVLGGKIAAWKDEDGDWYETGLHIFFGAYPNIQNLFG 161
MOMARDICA PDS                   LAGLSTAKYLADAGHKHVLLEARDVLGGKVAAWKDNDGDWYETGLHIFFGAYPNLQNLFG 173
CAROTENE 7,8 DESATURASE         LAGLSCAKYLADAGHTPFVYEARNVLGGKVAAWKDDDGDWYETGLHIFFGAYPNMLQLFK 69
PEA PDS                         ------------------------------------------------------------
ZETA CAROTENE DESATURASE        VAGLSAAIELVDRGHTVELYEKRKVLGGKVSVWKDSDGDSIESGLHIVFGGYTQLQKYLD 69

TNAU P                          ELGINDRLQWKEHSMIFAMPSKPGEFSRFDFSEALPAPLNGILAILKNNEMLTWPEKVKF 107
PHYTOENE DEHYDROGENASE          ELGINDRLQWKEHSMIFAMPSKPGEFSRFDFSEALPAPLNGILAILKNNEMLTWPEKVKF 240
GENTIAN PDS                     ELGINDRLQWKEHSMIFAMPNKPGEFSRFDFAEVLPAPLNGIWAILKNNEMLTWPEKVKF 236
CITRUS PDS                      ELGINDRLQWKEHSMIFAMPNKPGEFSRFDFPEVLPAPLNGILAILRNNEMLTWPEKVKF 209
ORYZA PDS                       ELGINDRLQWKEHSMIFAMPNKPGESSRFDFPETLPAPLNGIWAILRNNEMLTWPEKVKF 221
MOMARDICA PDS                   ELGINDRLQWKEHSMIFAMPNKPGEFSRFDFPEVLPAPINGIWAILRNNEMLTWPEKVKF 233
CAROTENE 7,8 DESATURASE         ELDIEDRLQWKSHSMIFNQPEEPGTYSRFDFPD-LPAPINGVAAILSNNDMLSWPEKISF 128
PEA PDS                         ----------KEHSMIFAMPSKPGQFSRFDFLEVLPSPLNGIWAILRNNEMLTWPEKIKF 50
ZETA CAROTENE DESATURASE        KIGAGDNYLWKDHSLIYAESD--GKQSFFKKAN-LPSPWAEVVGGLQADFLTMW-DKISL 125

TNAU P                          AIGLLPAMLGGQSYVEAQDGISVKDWMRKQGVPDRVTDEVFIAMSKALNFINPDELSMQC 167
PHYTOENE DEHYDROGENASE          AIGLLPAMLGGQSYVEAQDGISVKDWMRKQGVPDRVTDEVFIAMSKALNFINPDELSMQC 300
GENTIAN PDS                     AIGLVPAILGGQPYVEAQDGITVKDWMRKQGVPDRVTEEVFIAMSKALNFINPDELSMQC 296
CITRUS PDS                      AIGLLPAIIGGQAYVEAQDGLTVQEWMRKQGVPDRVTTEVFIAMSKALNFINPDELSMQC 269
ORYZA PDS                       ALGLLPAMVGGQAYVEAQDGFTVSEWMKKQGVPDRVNDEVFIAMSKALNFINPDELSMQC 281
MOMARDICA PDS                   AIGLLPAMLGGQPYVEAQDGLTVQEWMRNRGVPDRVTTEVFIAMSKALNFINPDELSMQC 293
CAROTENE 7,8 DESATURASE         GLGLVPAMLRGQNYVEDCDKYSWTEWLKKQNIPERVNDEVFIAMSKALNFIGPDEISSTV 188
PEA PDS                         AIGLLPAILGGQAYVEAQDGVSVKEWMRKQGIPERVTDEVFIAMSKALNFINPDELSMQC 110
ZETA CAROTENE DESATURASE        IKGLWPALAGNEEYFRSQDHMTYSEWHRLHGASEHSLQKLWRAIALAMNFIEPNVISARP 185

TNAU P                          ILIALNRFLQEKHGSKMAFLDGNPPERLCMPIG-------------------------- 200
PHYTOENE DEHYDROGENASE          ILIALNRFLQEKHGSKMAFLDGNPPERLCMPIVEHIESKGGQVRLNSRIKKIELNEDGSV 360
GENTIAN PDS                     ILIALNRFLQEKHGSKMAFLDGNPPERLCMPIADHIQSRGGEVRLNSRIQRIELNEDGSV 356
CITRUS PDS                      ILIALNRFLQEKHGSKMAFLDGNPPERLCLPIVEHIQSLGGEVRLNSRVQKIELNDDGTV 329
ORYZA PDS                       ILIALNRFLQEKHGSKMAFLDGNPPERLCMPIVDHVRSLGGEVRLNSRIQKIELNPDGTV 341
MOMARDICA PDS                   ILIALNRFLQEKHGSKMAFLDGNPPERLCEPVVEHIRSLGGEVRLNSRIQKIELNNDGTV 353
CAROTENE 7,8 DESATURASE         LLTALNRFLQEKNGSKMAFLDGAPPERLCQPIVDHIRTLGGDVFLNSPLKKINLKEDGSV 248
PEA PDS                         ILIALNRFLQEKHGSKMAFLDGNPPERLCMPIVDHIQSLGGEVHLNSRIKSIELNDDSTV 170
ZETA CAROTENE DESATURASE        ILTIFKYFGTDYAATKFAFFRKNPGDSMIEPMRQYIQSKGGRIFIDARLSRFELNDDKTI 245
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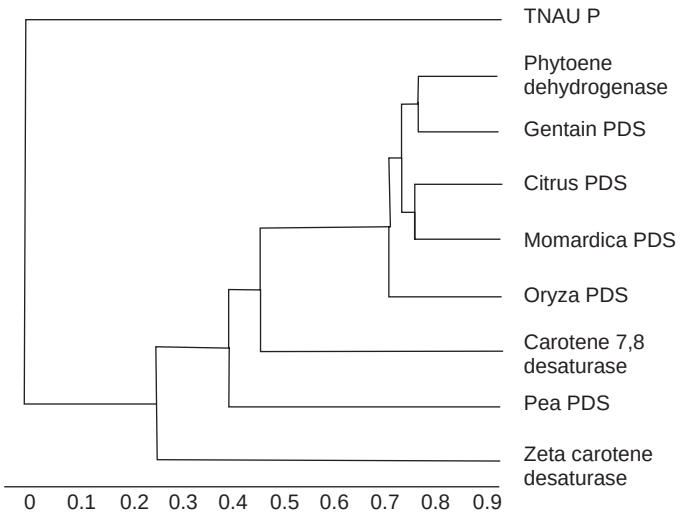

Fig. 4. Phylogenetic grouping of TNAU P with other carotenoid genes
TNAU P – *Phytoene destaurase* gene from *L. pimpinellifolium* LA 1593

Phytoene dehydrogenase	*Phytoene dehydrogenase* from *L. esculentum*
Gentian PDS	*Phytoene desaturase* gene from *Gentian lutea*
Citrus PDS	*Phytoene desaturase* gene from *Citrus sinensis*
Oryza PDS	*Phytoene desaturase* gene from *Oryza sativa*
Momardica PDS	*Phytoene desaturase* gene from *Momardica charantia*
Carotene 7,8 desaturase	Carotene 7,8 desaturase

digestion and PCR analysis (Fig. 2a, 2b). The confirmed recombinant clone was sequenced and named as TNAU P. The sequences were submitted in National Centre for Biotechnology Information (NCBI), New York, USA. The NCBI genebank accession number for the sequence corresponding to *Phytoene desaturase* gene, named TNAU P from *L. pimpinellifolium* is DQ911639. The high level of lycopene in the wild species, *L. pimpinellifolium* may be due to increased expression of the *PDS* gene. This view was also supported by the fact that the amplified product of *Phytoene desaturase* gene on agarose gel was more intense in the wild species, *L. pimpinellifolium* compared to that of other varieties, hybrids and *L. peruvianum*.

The sequence annotation results and BLAST analysis of cDNA clone TNAU P from *L. pimpinellifolium* LA 1593 revealed 99.6% homology to that of the already reported sequence of *L. esculentum, Phytoene desaturase* gene using BLAST software.

The nucleotide sequence of TNAU P was translated to protein sequence. The protein sequence of TNAU P showed 99% identity with already available *Lycopersicon esculentum Phytoene desaturase* gene using NCBI-BLASTp software. The multiple sequence alignment of TNAU P showed considerable homology with *Phytoene dehydrogenase*, *carotene 7, 8 desaturase*, *zeta carotene desaturase* and other *Phytoene desaturase* genes from Gentian, *Oryza*, *Momardica*, citrus, pea using DBClustal software (Fig. 3). The results are in agreement with Li *et al.* (1996), Linden *et al.* (1994), Pecker *et al.* (1996) and Bartley (1991) who independently reported the homology and identity of available dicot and monocot *Phytoene desaturase* gene sequences. Phylogenetic tree constructed using Treetop software with the multiple sequence alignment data showed two clusters with grouping of TNAU P as one cluster and other desaturase genes in another cluster (Fig. 4). The phylogenetic relationships between various carotene desaturases has been extensively reviewed by Sandmann (1994). Li *et al*. (1996) reported a similar phylogenetic relationship of maize *PDS* with available monocot and dicot amino acid sequences and concluded that they have some evolutionary relationships.

Through quality parameters and gene isolation studies, it could be concluded that *L. pimpinellifolium LA* 1593 is a potential source for lycopene and it could be further exploited for isolation of lycopene specific genes.

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