

Variation in *Lycopene Epsilon-Cyclase* Gene in Selected Tomato Genotypes Revealed by EcoTILLING and Sequencing

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Selected tomato genotypes with contrasting fruit colors of orange and red were investigated for sequence-level variations of candidate genes involved in lycopene cyclization. Sequence-specific markers for tomato *lycopene beta-cyclase* (3) and *lycopene epsilon-cyclase* (1) genes were designed and used to screen for putative single nucleotide polymorphisms (SNPs) through EcoTILLING and Sanger sequencing. Despite being regarded as among the evolutionarily conserved genes in the carotenoid biosynthetic pathway of tomato, four homozygous and heterozygous SNPs were identified in *lycopene epsilon-cyclase* gene at the upstream of Exon 1 (1 SNP) and the intronic region between Exons 1 and 2 (3 SNPs) based on multiple sequence alignment of the processing tomato hybrid ‘Ilocos Red’ and table type inbred ‘Hawaii7996’. These SNPs may have a regulatory association with variations in tomato carotenoid metabolism. Interestingly, no sequence difference was found between FLA456 and ‘Super Apollo’ despite being characterized by orange and red fruit colors, respectively. The results support prior studies suggesting that *lycopene cyclase* genes are transcriptionally controlled as evidenced by their highly conserved sequences. The SNPs characterized in this study at the promoter and intronic regions of *lycopene epsilon-cyclase* are starting loci to investigate further the genetic control of this gene in regulating carotenoid metabolism and products that result in varying tomato fruit phenotypes.

Keywords: carotenoid genes, EcoTILLING, *lycopene epsilon-cyclase*, SNP, tomato

INTRODUCTION

The tomato carotenoid biosynthetic pathway is extensively studied, and its gene and gene products have already been characterized (Cunningham and Gantt 1998; Isaacson *et al.* 2004). Lycopene cyclization is an important branch step in the carotenoid biosynthesis pathway since regulating these branch points directly affects carotenoid composition

(Liu *et al.* 2015). The *lycopene beta-cyclase* is one of the crucial enzymes of the carotenoid biosynthetic pathway. Tomato has two types of *lycopene beta-cyclase* genes corresponding to two different enzymes – the chloroplast-specific *Lcy-b* and chromoplast-specific *CYC-B* (Dalal *et al.* 2010). The action of enzymes lycopene epsilon-cyclase (*Lcy-e*) and chloroplastic lycopene beta-cyclase (*Lcy-b*) converts the red pigment all-*trans*-lycopene to yellow pigment α -carotene, whereas the chloroplastic (*Lcy-b*) or

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chromoplastic (CYC-B) lycopene beta-cyclase enzyme synthesizes it into orange β -carotene pigment (Alós *et al.* 2019). These carotenoid pigments in tomatoes, particularly lycopene and β -carotene, are recognized to have major antioxidant and anti-cancer activities (Stahl and Sies 1996; Raiola *et al.* 2014) and, thus, fruit color directly indicates the nutritional and functional value (Martí 2016).

TILLING (Targeting-Induced Local Lesions IN Genomes) is a reverse genetics strategy that combines mutation induction and gene-specific digestion-based assay to identify novel genetic variation and investigate gene function. EcoTILLING (Ecotype Targeted Induced Local Lesions IN Genome) follows a similar strategy but utilizes natural populations to discover natural genetic variants and putative gene function (Barkley and Wang 2013). This strategy enables efficient screening of a gene of interest across several samples to detect naturally occurring INDELs (short insertions and deletions) and/or SNPs (single nucleotide polymorphisms) and other DNA polymorphisms such as differences in satellite repeat number (Till *et al.* 2006). EcoTILLING can also be used to determine heterozygosity levels of a gene fragment in highly heterozygous outcrossing species (Gilchrist and Haughn 2005). By eliminating identical haplotypes, SNP discovery and polymorphism identification may be implemented rapidly without the need to sequence every individual in a population. In addition, EcoTILLING can detect multiple polymorphisms in a single fragment because CEL I endonuclease will digest only a small proportion of heteroduplexes or can be used to survey rare DNA polymorphisms (Till *et al.* 2006).

In this study, we analyzed the gene sequences of enzymes responsible for lycopene cyclization in candidate elite breeding materials, economically important tomato cultivars, and other genotypes to investigate whether natural polymorphism coexists with red and orange fruit color phenotypes. Putative allelic differences, specifically SNPs, present in the homologs and a paralog of *lycopene beta-cyclase* gene and *lycopene epsilon-cyclase* gene were identified using EcoTILLING and Sanger sequencing methods and later compared between genotypes with different fruit colors. Sequence variation presented in this study can be further investigated to assess its segregation patterns and understand gene expression regulating carotenoid metabolism and its corresponding range of phenotypic variation.

MATERIALS AND METHODS

Plant Materials

Five (5) tomato breeding lines consisting of table-type and processing varieties were selected on the basis of fruit color, fruit quality, and resistance to pests and diseases. Genotypes included in the study are as follows: *S. lycopersicum* [a] tomato table type varieties – FLA456, ‘Super Apollo’ and ‘Hawaii 7996’; [b] processing tomato ‘Ilocos Red’; and [c] wild tomato relative, *S. pimpinellifolium*, ‘West Virginia 700’.

The tomato cultivar FLA456 is a beta-carotene-enriched tomato that bears orange-colored fruits and is developed by the World Vegetable Center (WorldVeg) (formerly Asian Vegetable Research and Development Center or AVRDC). ‘Ilocos Red’ is a processing-type cultivar with deep red fruit color that is commonly utilized by Northern Foods Corporation (NFC) to process into a paste for local consumption (PCAARRD 2018). ‘Super Apollo’ is a table-type cultivar that is open-pollinated and considered a farmer’s variety. The ‘Super Apollo’ variety used in this study is an improved strain or selection by the Institute of Plant Breeding and is characterized also by a deep red fruit color. ‘Hawaii 7996’ is a breeding line developed at Hawaii University for bacterial wilt resistance (Scott *et al.* 2004), whereas ‘West Virginia 700’ is a wild relative characterized by late blight resistance (*Ph2*) (Moreau *et al.* 1998). ‘Hawaii 7996’ and ‘West Virginia 700’ both have red fruit color.

Gene Targets and Sequence Validation

The genes encoding for *lycopene beta-cyclase* and *lycopene epsilon-cyclase* were selected as targets. These are key enzymes in the conversion of lycopene into α - and β -carotene, respectively. A total of four gene homologs were identified based on the tomato reference genome Heinz 1706 version SL3.0 from the Sol Genomics Network (SGN, (<https://solgenomics.net/>)) – specifically, three for *lycopene beta-cyclase* [namely, *lycopene beta-cyclase* (*CrtL-b*), *lycopene beta-cyclase2* (*CrtL-b2*), and *beta-lycopene cyclase* (*CrtR-b*)] and one for *lycopene epsilon-cyclase* (*CrtL-e*), as presented in Table 1.

Gene-specific primers were designed to amplify regions of tomato *lycopene epsilon-cyclase* and *lycopene beta-cyclase* genes (Table 2).

DNA Extraction, Quantification, Pooling, and Amplification

Young leaf samples from 10 random individual plants 30 DAS were sampled per tomato genotype. Genomic DNA was isolated using the CTAB protocol with minor modifications (Bernatzky and Taksley 1986; Fulton *et*

Table 1. Tomato carotenoid genes used to develop sequence-specific markers for the SNP screening analysis in this study.

Gene	Locus symbol	Locus name	SolGenomics ID	Base pairs (bp)	No. of exons	Chromosome location
<i>CrtL-b</i>	<i>crtl1, Lcy-b</i>	<i>lycopene beta-cyclase</i>	Solyc04g040190.1	1,503	1	4
<i>CrtR-b</i>	<i>b, CYC-B</i>	<i>Beta-lycopene cyclase</i>	Solyc06g074240.3.1	1,497	1	6
<i>CrtL-b2</i>	<i>Lcy-b2</i>	<i>lycopene beta-cyclase2</i>	Solyc10g079480.1	1,503	1	10
<i>CrtL-e</i>	<i>Delta, Lcy-e</i>	<i>lycopene epsilon cyclase</i>	Solyc12g008980.1	4,956	11	12

Table 2. Primer sets designed to target each tomato *lycopene beta-cyclase* gene (homologs *CrtL-b* and *CrtL-b2*, and paralog *CrtR-b*) and *lycopene epsilon-cyclase* gene (*CrtL-e*).

Gene, solgenomics ID	Locus name and symbols	Primer name	Sequence	Product length in base pairs (bp)	Regions covered	Optimal Ta (°C)
<i>lycopene beta-cyclase</i> (Solyc04g040190)	<i>crtl1, Lcy-b, CrtL-b</i>	CrtL-b1	F: TCGACACACTAATGCAAAGAAAACA R: AATCTCGCCAATCCATGAAAACC	1,259	Single exon	54
		CrtL-b2	F: GCTTATGGCATTGCTGAAG R: TGTGAGAGATAGAGCAAGCAGAG	935		58
<i>Beta-lycopene cyclase</i> (Solyc06g074240)	<i>b, CYC-B, CrtR-b</i>	CrtR-b1	F: ACTGGGATTGGGAAAAGGGTAAA R: TATGCACACAAGTCATAGGCCAT	688	Single exon	58
		CrtR-b2	F: TGGTGTGTTGGTTGATGAGTTTG R: GACACAAGCTGAGTAAACCAAGT	1,146		54
		CrtR-b3	F: TCCTAAATACTGGCAAGGGTTCC R: GGAAAGAGGGCCATTGACAGATA	453		58
<i>lycopene beta-cyclase2</i> (Solyc10g079480)	<i>Lcy-b2, CrtL-b2</i>	CrtL-b21	F: CCACAAGCATATCCTTTACCAGG R: CACACTGCTCATCTTCTTCA	1,267	Single exon	54
		CrtL-b22	F: CAACAGTGGTCTTGATGCGACG R: CGAAAGGGAGAAGTTTCTCATCA	1,111		55
<i>lycopene epsilon-cyclase</i> (Solyc12g008980)	<i>Delta, del, Lcy-e, CrtL-e</i>	CrtL-e1	F: GAAATAACTCCAGCAAATCAGATCC R: GACACAAGTATCTCTATCTCCAGA	1,292	5' UTR and Exon 1	54
		CrtL-e2	F: TATCAAGGAGGAAGAAGCGGAG R: TATCCC GCCAAACATGTTCAATG	1,379	Exons 2 and 3	60
		CrtL-e3	F: GTTACATGCATGAACACAGGGTC R: CAGATCGAATGGCATTGCATCTT	1,234	Exons 3, 4, 5, 6 and 7	60
		CrtL-e4	F: GCTCCACACATTACTACAATTGGC R: CTGCCTCAGTTTCAATGTTAC	1,119	Exons 7, 8, 9 and 10	54
		CrtL-e5	F: GCAGCTGGATATTGAGGGGATAA R: TGAACAGCTCAGAGTCATGCAC	1,227	Exon 10 and 11	62

al. 1995). DNA quality and yield were assessed through agarose gel electrophoresis using 1% agarose in 1X Tris-boric EDTA (TBE) running buffer. Individual DNA samples were normalized into 50ng/uL concentration and assigned into pools of 10.

The PCR was performed using the following conditions: initial denaturation at 94 °C (5 min), 35 cycles of 95 °C

(30s) denaturation, 54–62 °C annealing (1 min), and 72 °C (2 min) extension, followed by one cycle at 72 °C (7 min) final extension. Each 20-μL PCR reaction consisted of 100 ng genomic DNA, 1X PCR buffer, 2.5 mM MgCl₂, 0.2 mM dNTPs (Amersham Pharmacia Biotech AB, Uppsala, Sweden), 0.2 μM each of forward and reverse primer and 1U *Taq* DNA polymerase (Vivantis Technologies).

Amplification was carried out in T100™ Thermal Cycler (Bio-rad Laboratories Inc., Hercules, CA, USA).

Agarose-based EcoTILLING Analysis using *CEL 1*

EcoTILLING using crude extract of *CEL 1* endonuclease enzyme extract isolated from tomato followed the procedure of Alcasid and colleagues (2019) with modifications. The tomato breeding line FLA456 was assigned as the reference sample, whereas ‘Super Apollo’ and ‘Ilocos Red’ were assigned as test samples owing to fruit color classes orange and red, respectively. ‘West Virginia 700’ and ‘Hawaii 7996’ were used as control genotypes.

The FLA456 amplicon was mixed with an equal volume of each test tomato sample and control genotype. Homoduplex controls were also prepared for each sample genotype. PCR products and PCR product mixes were subjected to heteroduplex formation as a preparatory procedure for the succeeding endonuclease digestion. Heteroduplex formation was carried out in a thermal cycler with the following temperature profile: 10 min of 95 °C denaturation, followed by a temperature step-down from 95 to 85 °C with a decrement of 2.0 °C/s, and from 85 °C further temperature step-down to 25 °C at a decrement of 0.3 °C/s held for 1 min for every 10 °C-step-down (*i.e.* 85 °C, 75 °C, 65 °C, and so on); and final storage of 12 °C.

Heteroduplexed PCR products were each added with an endonuclease mixture containing 8.3 µL distilled water, 1.5 µL 10X CJE Buffer and 0.2 µL crude *CEL 1* endonuclease. The mixture was then incubated at 45 °C for 45 min. Digested products were immediately placed on ice and added with 5 µL of 0.015 M EDTA to stop the enzymatic reaction.

Digested PCR products were resolved in 2% agarose gel containing 1 µL GelRed (Biotium, Hayward, CA), and subjected to electrophoresis at 80 V for 40 min in 1X TBE buffer and visualized using the ENDURO™ GDS gel documentation system (Labnet International, Inc.).

Sequence Analysis

Three (3) biological replicates of amplicons that exhibited putative SNPs inferred through agarose EcoTILLING were sent for standard capillary sequencing (Macrogen, Inc., Seoul, Korea). Quality control and assembly of the consensus sequence for each individual were inferred using Geneious Prime (version 2021.1.1; Biomatters Ltd.). Homozygosity and heterozygosity of SNPs were deduced from the number of peaks at a particular base position with a high-quality base call. A single peak denotes homozygosity while double peaks signify heterozygosity.

Cis-regulatory element motifs adjacent to SNP were identified using PLACE (PLAnt Cis-acting regulatory

DNA Elements) software (<https://www.dna.affrc.go.jp/PLACE/?action=newplace>) (Higo *et al.* 1999). For cluster analysis, multiple sequence alignment was executed using ClustalW (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) implemented in the MEGA11 software ver. 10.1.7 (Tamura *et al.* 2021), followed by tree construction using the neighbor-joining (NJ) method with 1000 bootstrap replicates and a maximum composite likelihood model with uniform rates among lineages.

RESULTS AND DISCUSSION

EcoTILLING Using *CEL 1* Revealed Putative SNPs in *CrtL-e*

The EcoTILLING screening was done to mine potentially relevant SNPs among populations and investigate their coexistence in red and orange fruit color phenotypes. Among primer pairs used to study the four genes of interest, only amplicons with *CrtL-e1* revealed potential nucleotide sequence variations among genotypes due to the distinct *CEL 1* digestion profiles. The positive digestion patterns for the heteroduplex and homoduplex lanes were unexpected since these genotypes are anticipated to have limited genetic diversity, owing to the narrow genetic base of cultivated tomatoes (Alvarez *et al.* 2001).

Digestion with crude *CEL 1* endonuclease revealed that amplicon *CrtL-e1*, which spans the first exon of the *lycopene epsilon-cyclase* gene and its immediate flanking region, was found to have mismatch sites from which probable natural SNPs among the population (Figure 1). The presence of band patterns with smaller fragment sizes exhibits weak but visible signals in the digestion profiles of the heteroduplexed samples with FLA456. These genotypes are ‘Ilocos Red’, Hawaii 7996’, and ‘West Virginia 700’. ‘Ilocos Red’ (Lane 2) exhibited roughly 500 bp, 250 bp, and light smears of less than 200 bp *CEL-1* digestion fragments similar to the pattern of the heteroduplexed sample of ‘Hawaii 7996’ (Lane 5). In Lane 4, FLA456-‘West Virginia 700’ has observed patterns with approximately 850 and 650 bp digested DNA fragments in size (Figure 1, Group A). Digestion fragments are also present from the homoduplexed individual plant samples for FLA456 (Lane 6) with 850bp fragment size, as well as ‘Ilocos Red’ (Lane 7) and ‘West Virginia 700’ (Lane 9) with similar fragment sizes as its heteroduplexed counterpart. The intense, long smear bands near higher molecular mass from homoduplexed samples of ‘Hawaii 7996’ (Lane 10) (Figure 1) appear to have incomplete *CEL 1* digestion patterns. The presence of mismatches that *CEL 1* cleaved should be within the base difference occurring between individual plant samples. However, there is also a possibility that the high concentration of

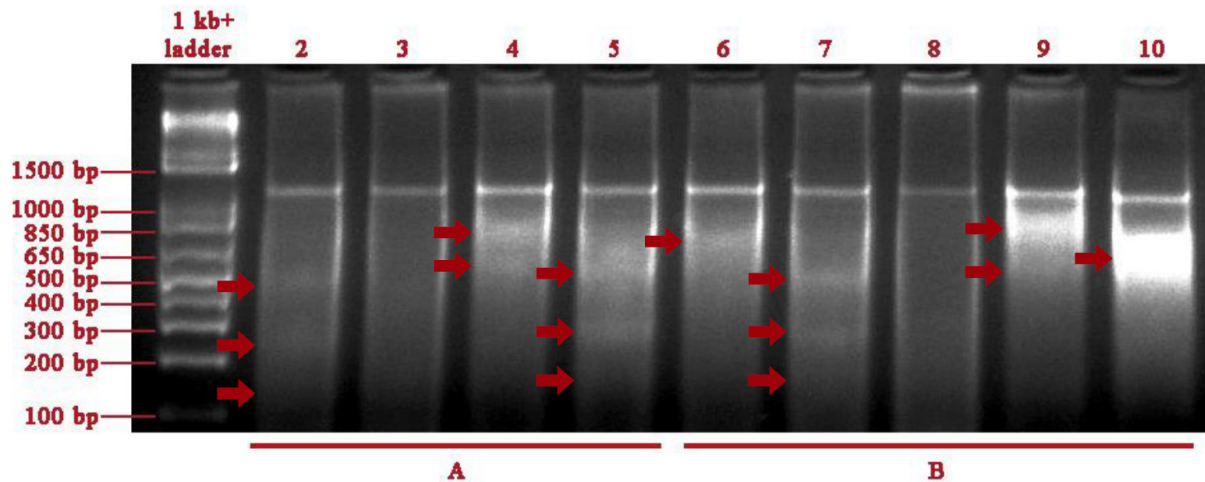


Figure 1. Agarose gel electrophoresis of CEL 1-digested amplicons of CrtL-e1. Group A includes CEL 1 digests of amplicon heteroduplexes of FLA456-‘Ilocos Red’ (Lane 2), FLA456-‘Super Apollo’ (Lane 3), FLA456-‘West Virginia 700’ (Lane 4), and FLA456-‘Hawaii 7996’ (Lane 5). Lanes 6–10 (group B) are endonuclease digests of homoduplex controls FLA456, ‘Ilocos Red’, ‘Super Apollo’, ‘West Virginia 700’ and ‘Hawaii 7996’, respectively. The digested fragments are marked with red arrows.

the crude CEL 1 enzyme may have contributed to the accumulation of mismatched cleavage products in a form of PCR product networks that had remained annealed, resulting in the appearance of smears or as a result of incomplete digestion of pooled DNA template (Till *et al.* 2004). In comparison, no digestion products were found in homoduplex profiles of ‘Super Apollo’ and FLA456 (Lane 3) and its homoduplex control (Lane 8), denoting that there is no sequence difference between FLA456 and ‘Super Apollo’ from this particular region of the genome.

Sequence Confirmation of Putative SNPs

To confirm the presence and determine the exact position of putative SNPs cleaved by CEL 1 endonuclease, amplicons of CrtL-e1 were sent for bidirectional sequencing. The forward and reverse sequences have good quality scores and clean chromatogram peaks. After further quality control, only a total of 1245 bp sequence of *lycopene epsilon-cyclase* gene of three random individual plants from each of the five genotypes in the study was processed for further analysis. The ClustalOmega alignment (Figure 2) and chromatograms from sequence data revealed unique SNPs from ‘Ilocos Red’ and ‘Hawaii 7996’ separating them apart from other genotypes in the study. The SNPs found are located before the start site of *lycopene epsilon-cyclase* gene and within the intronic region of Exons 1 and 2. The presence of these SNPs was further confirmed by multiple sequence alignment as summarized in Table 3.

The presence of around 500 bp and 250 bp fragments from digestion products of CEL 1 (Figure 1) in the heteroduplexed samples of FLA456 and ‘Ilocos Red’ and

between FLA456 and ‘Hawaii 7996’ confirms the presence of the 2 SNPs found at positions 271 and 768 relative to the sequenced amplicon. Although very faint and diffused, visible fragment patterns of molecular weights less than 200 bp represented SNPs in positions 982 and 1094. The similarity in fragment size of homoduplexed samples among ‘Ilocos Red’ population successfully detected the presence of heterozygous SNPs within individual plant samples.

There are no SNPs found along *lycopene epsilon-cyclase* gene of ‘Super Apollo’, wild close relative *S. pimpinellifolium*, ‘West Virginia 700’, and FLA456 when comparing within the three replicates from individual samples and among genotypes despite the presence of CEL 1 digestion fragments. The ‘West Virginia 700’ is a wild relative of the tomato, and it is possible that SNPs, as indicated by the digestion products (Lanes 4 and 9, Figure 1) were present within the population but were undetected due to the random selection of samples that were sent for Sanger sequencing. The same explanation can be inferred with light smears in the homoduplex product of FLA459 with a long history of crossing as breeding material. However, it is highly likely that these fragments are due to the incomplete digestion of PCR products, which remained annealed since the agarose gel used has no denaturing agent (Till *et al.* 2004) and because sequencing results are proven to be a much more reliable basis for the confirmation of the SNPs. As mentioned, limited to no sequence differences are expected since these genes are highly conserved. Earlier studies also reported the limited number of SNPs in *lycopene beta-cyclase* (CYC-B) and overall lower genomic diversity in tomatoes

Table 3. Heterozygous and homozygous SNPs (italicized) found in the *lycopene epsilon-cyclase* gene. The SNP location, indicated as numbers, is the relative position of the SNPs to the processed consensus sequence. The type of mutation is also indicated as either transversion or transition.

Sample	SNP location			
	271	768	982	1094
‘Ilocos Red’	<i>T</i>	<i>R (A/G)</i>	<i>K (G/T)</i>	<i>W (A/T)</i>
‘Hawaii 7996’	<i>T</i>	<i>G</i>	<i>T</i>	<i>A</i>
‘West Virginia 700’	<i>A</i>	<i>A</i>	<i>G</i>	<i>T</i>
‘Super Apollo’	<i>A</i>	<i>A</i>	<i>G</i>	<i>T</i>
FLA456	<i>A</i>	<i>A</i>	<i>G</i>	<i>T</i>
‘Heinz 1706’*	<i>A</i>	<i>A</i>	<i>G</i>	<i>T</i>
SNP position	Upstream of exon 1	Intronic**	Intronic**	Intronic**
Type of mutation	Transversion	Transition	Transversion	Transversion

*Sequence form SolGenomics Database

**Intronic regions refer to the position between exon 1 and exon 2 of *lycopene epsilon-cyclase* gene.

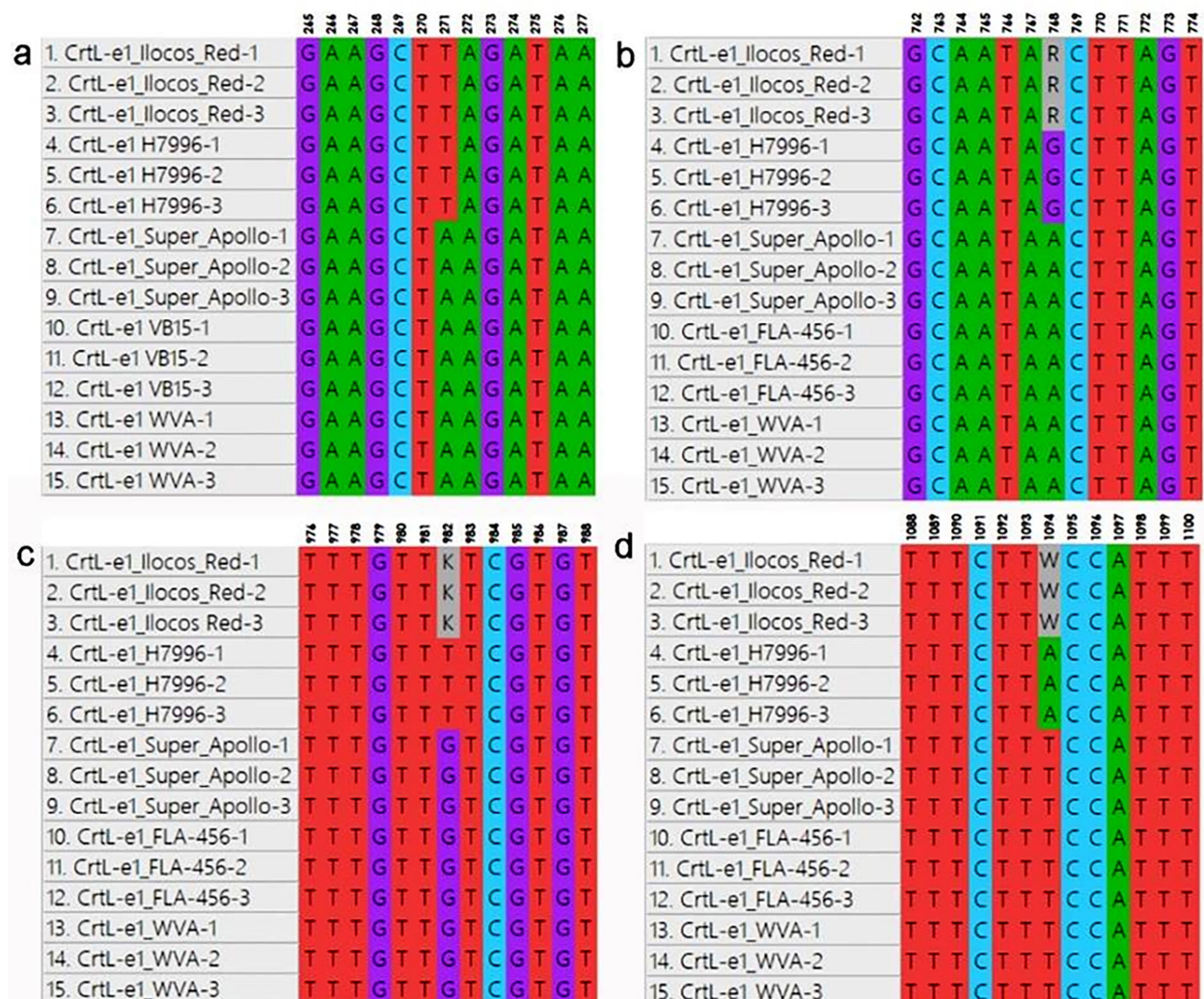


Figure 2. Multiple sequence alignment of tomato varieties in the study. SNPs from ‘Ilocos Red’ and ‘H7996’ were indicated with color differences. Locations are relative to the 1245bp assembled and concatenated *CrtL-e1* amplicon sequence.

(Mohan *et al.* 2016a, b). For the beta-carotene enriched tomato breeding line FLA456, it is highly possible that the overexpression of genes involved in lycopene cyclization is due to transcriptional upregulation. This is in line with the findings of Pecker *et al.* (1996) and Ronen *et al.* (1999), stating that transcriptional regulation plays a major role in the expression of *lycopene beta-cyclase* and *lycopene epsilon-cyclase* genes in tomatoes.

The unique SNPs found within the ‘Ilocos Red’ and ‘Hawaii 7996’ amplicons are interesting to study, as they may indicate novel mutations that possibly become detectable in frequency but are not fixed in a population or an alternative allele affecting gene expression of the *lycopene epsilon-cyclase* gene.

The first confirmed SNP was identified along the 5'UTR of *lycopene epsilon-cyclase* gene within ‘Ilocos Red’ and ‘Hawaii 7996’ (Figure 2a). In ‘Ilocos Red’, three heterozygous SNPs were found between the intronic region of Exons 1 and 2 of the *lycopene epsilon-cyclase* gene, whereas homozygous SNPs were found within the same position in ‘H7996’ (Fig 2bcd). Transversion is the dominant type of mutation or substitution pattern in the SNPs found in *CrtL-e*, which is consistent with findings in *lycopene beta-cyclase gene (CYC-B)* in tomatoes (Mohan *et al.* 2016a). This occurrence may be associated with the methylation pattern dictating the binding of transcription factors near the promoter region, which controls ripening through the upregulation of the carotenoid biosynthetic pathway when the promoter is in a demethylated state (Mohan *et al.* 2016a; Zhong *et al.* 2013).

The SNPs found within the processing tomato variety ‘Ilocos Red’ are mostly heterozygous as expected for a hybrid variety. Allele-specific expression of these heterozygous SNPs is interesting to be investigated further, as expression imbalance may play a role in the phenotypic variation. Many studies have suggested that the intronic region of a gene plays an important regulatory role (Lareau *et al.* 2004). Thus, these SNPs found within these regions may or may not have an influence on gene splicing that may result in a different mRNA variant.

Regulatory Elements near Identified SNPs

Since the site of SNPs constitutes noncoding DNA regions, candidate motifs associated with *cis*-regulatory DNA elements (CREs) were identified \pm 20 bases of each SNP to identify potential recognition sites of known transcriptional elements. These CREs may influence mRNA translation affecting levels of expression of the gene of interest. For the SNP found along the 5'UTR of *lycopene epsilon-cyclase* gene is located +25 upstream near the translational start site, the most represented motif found is *DOFCOREZM* (5'-AAAG-3'). *DOFCOREZM*

is the binding site for Dof proteins, which play a number of functions in the regulation of plant processes like photosynthesis and light-regulated gene expression among others (Yanagisawa 2002). On the other hand, motifs found downstream of the SNP are associated with *GATABOX* (5'-GATA-3'), *GTICONSENSUS* (5'-GRWAAW-3'), and *IBOXCORE* (5'-GATAA-3'). *GATABOX* is a motif in CaMV 35S promoter, whereas both *GTICONSENSUS* and *IBOXCORE* are both conserved sequence binding sites for light-regulated genes in monocots and dicots (Wan *et al.* 2007). Other CREs found are *PYRIMIDINEBOXOSRAMYIA* (5'-GATA-3'), *PIBS* (5'-GNATATNC-3'), *MYBST1* (5'-GGATA-3'), and *CACTFTPPCA1* (5'-YACT-3').

For the SNPs detected within the intronic region, the most commonly represented motif are *DOFCOREZM* (5'-AAAG-3'), *IBOXCORE* (5'-GATAA-3'), *GTICONSENSUS* (5'-GRWAAW-3'), and *GATABOX* (5'-GATA-3'), which are all found in the third and fourth SNPs. Also identified along the mentioned SNPs are *POLLENILELAT52* (5'-AGAAA-3'), which is one of the two co-dependent regulatory (the other one is *LECPLEACS2*) signal element responsible for pollen-specific expression (Bate and Twell 1998) and *ARRIAT* (5'-NGATT-3') or the “ARR1-binding element,” which is found in *Arabidopsis* as a response regulator (Sakai *et al.* 2000).

For the second SNP identified, the regulatory elements identified are unique such that it is not found around other SNPs identified in the study. These elements are *MYBCORE* (5'-CNGTTR-3'), *MYB2CONSENSUSAT* (5'-YAACKG-3'), *MYBCOREATCYCBI* (5'-AACGG-3'), *E2FCONSENSUS* (5'-WTTSSCSS-3'), *CAATBOX1* (5'-CAAT-3'), *ANAERO2CONSENSUS* (5'-AGCAGC-3'), and *ROOTMOTIFTAPOX1* (5'-ATATT-3'). It is interesting to note that regulatory elements responsible for the activation of stress response genes were identified, which are *MYBCORE* and *MYB2CONSENSUSAT*. Characterized in *Arabidopsis*, *MYBCORE* is a binding site for MYB plant proteins which controls the expression of genes that respond to water stress, whereas *MYB2CONSENSUSAT* is located in the promoters of the dehydration-responsive gene *rd22* and other genes (Abe *et al.* 2003).

In the third SNP, unique CREs found are *NODCONIGM* (5'-AAAGAT-3'), *OSEIROOTNODULE* (5'-AAAGAT-3'), *CACTFTPPCA1* (5'-YACT-3'), *TBOXATGAPB* (5'-ACTTTG-3'), *RAVIAAT* (5'-CAACA-3'), and *LTRECOREATCOR15* (5'-CCGAC-3'). For the fourth SNP, uniquely identified regulatory factors are *SEF4MOTIFGM7S* (5'-RTTTTTTR-3') and *TATABOX5* (5'-TTATTT-3').

Limited Gene Diversity in Lycopene Cyclase Genes

Tomato exhibits a narrow genetic base due to homozygosity brought about by domestication (Bai and Lindhout 2007). This manifests in the limited genetic diversity in cultivated varieties and difficulty in identifying informative molecular markers that distinguish among cultivars (Zhou *et al.* 2015). This is concurred by the results of the cluster analysis using the NJ method (Figure 3). The NJ tree demonstrated that the sequenced *lycopene epsilon-cyclase* gene region of two cultivated varieties (*i.e.* FLA456 and ‘Super Apollo’) formed a single branch with the wild relative *S. pimpinellifolium* (WVA). Despite the separation of ‘Ilocos Red’ and ‘H7996’ varieties, the genetic distance is minuscule. The grouping of the cultivated tomato to the wild relative *S. pimpinellifolium* was also reported in another study, which investigated the evolutionary relationships in the *CYC-B* gene sequence in 58 tomato accessions (Mohan *et al.* 2016a).

and breeding line ‘H7996’. Most of the SNPs found within ‘Ilocos Red’ are heterozygous, suggesting possible expression imbalance. SNP found within the 5’UTR region may be associated with the methylation pattern, which controls the upregulation of the *lycopene epsilon-cyclase* gene and, consequently, fruit ripening – whereas SNPs in intronic regions may or may not have influence with translational regulation resulting from internal or external stimuli such as light, stress, and fruit ripening. SNP markers can be developed to target heterozygous SNPs revealed in this study to further investigate carotenoid gene diversity in a larger tomato population. Generating a segregating population from crossing ‘Hawaii 7996’ and ‘FLA456’, as well as ‘West Virginia 700’ and ‘FLA456’, is also recommended to study the segregation patterns of the heterozygous SNPs and its corresponding range of phenotypic variance investigated using advanced phenotyping tools. Furthermore, the transcript expression of these genes should be investigated further in future studies.

CONCLUSION

Despite the highly conserved nature of the selected carotenoid genes, SNPs were identified along the 5’ UTR and intronic region between Exons 1 and 2 of *lycopene epsilon-cyclase* gene within processing type ‘Ilocos Red’

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This research was conducted through the funds provided by the DOST-PCAARRD (Department of Science and Technology–Philippine Council for Agriculture, Aquatic,

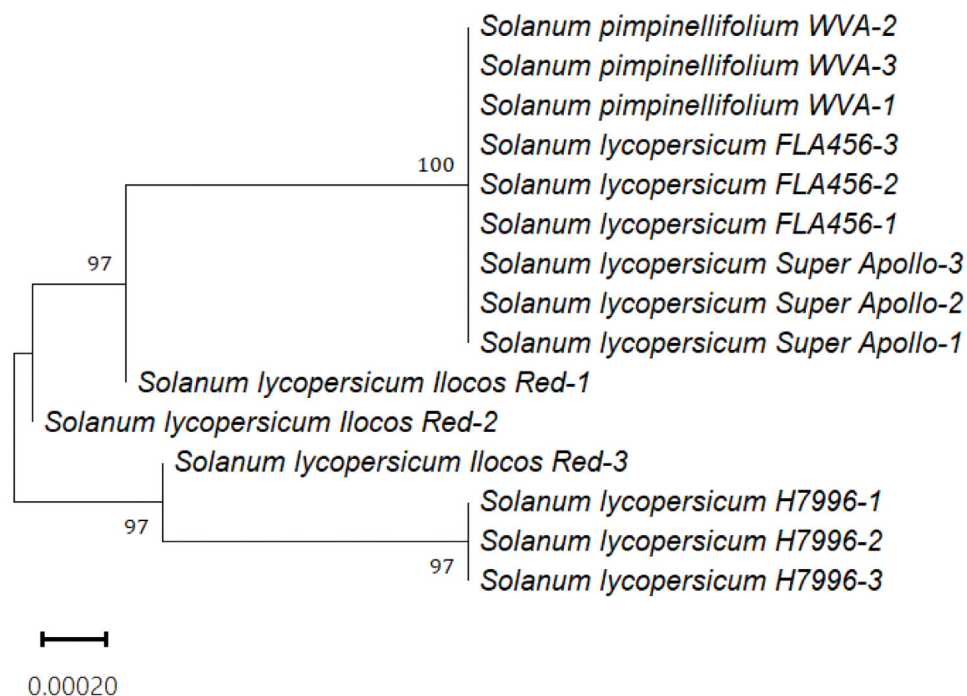


Figure 3. NJ tree of selected cultivated tomato varieties and wild relative, *S. pimpinellifolium* based on 1279 bp concatenated sequences covering the first exon and intronic region between exons 1 and 2 of tomato *lycopene epsilon-cyclase*. Next to the branches are the percentage of duplicate trees in which the related taxa clustered together in the bootstrap test (1000 repetitions).

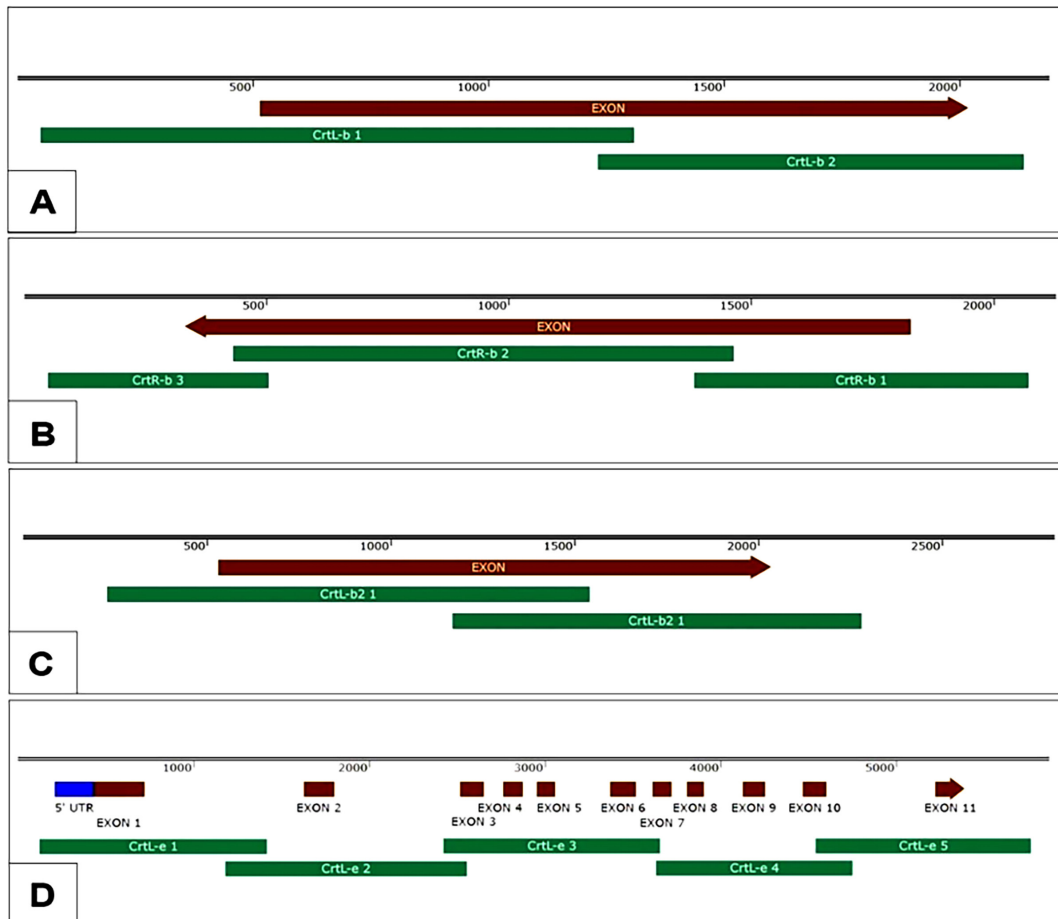
and Natural Resources Research and Development) under the GREAT (Graduate and Education Assistantship for Technology) Program. We gratefully acknowledge the efforts of Mr. Ronilo M. Bajaro and Mr. Melvin J. Malison for the technical assistance during laboratory experiments and greenhouse maintenance, respectively.

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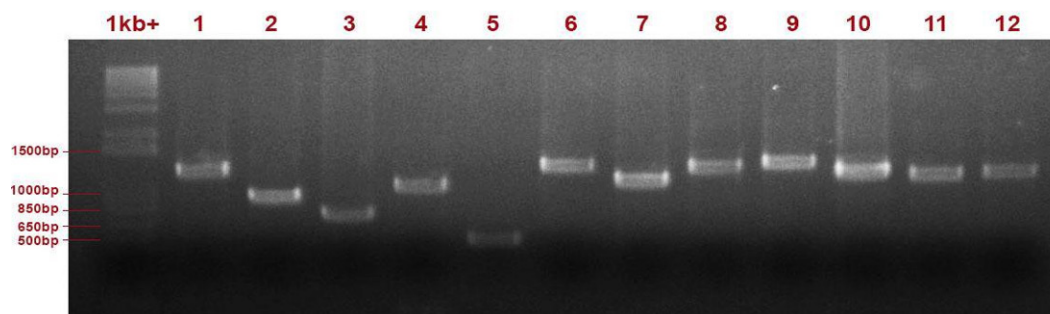
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APPENDICES



Appendix Figure I. Graphical representation of the genomic (coding) regions of tomato genome amplified by the different primer pairs for the following carotenoid genes: (A) chloroplastic *lycopene beta-cyclase* (*CrtL-b*) (Solyc04g040190.1); (B) chromoplasmic *lycopene beta-cyclase* (*CrtR-b*) (Solyc06g074240.3.1); (C) *lycopene beta-cyclase 2* (*CrtL-b2*) (Solyc10g079480.1); and (D) *lycopene epsilon-cyclase* (*CrtL-e*) (Solyc12g008980.1). Generated using SnapGene software using sequences and gene models obtained from the SGN database (<https://solgenomics.net/>). The red arrow bars represent the exons, while the green bars represent the target amplicons of each primer pair.



Appendix Figure II. Representative agarose gel electrophoresis of amplicons generated using the optimized PCR annealing temperatures (T_a) of the final set of primers for the four tomato *lycopene cyclase* genes. Using representative tomato variety FLA456, Lanes 1–12 are PCR products with the order of primers pairs, as listed in Table 2.

CLUSTAL O(1.2.4) multiple sequence alignment

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Solanum_lycopersicum_ilocos_Red-3      AAATCAGATCCCTCCACGTGCTGTTCATGAAATAAAAAATGTTACACATCAGCAAAAAA 60
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Solanum_lycopersicum_H7996-2          AAATCAGATCCCTCCACGTGCTGTTCATGAAATAAAAAATGTTACACATCAGCAAAAAA 60
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Solanum_pimpinellifolium_WVA-3        CTTCCTCCCTCCCTATCCGTATAAATTTATTCCTCTGTTTTCTCCATCCAAAAAAAAC 120
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Solanum lycopersicum_Super_Apollo-2	GAAAGTAAAATGTAATAGCAGTAGTGGTAGTGACAGTTGTGTAGTTGATAAAGAGATTT	480
Solanum lycopersicum_Super_Apollo-3	GAAAGTAAAATGTAATAGCAGTAGTGGTAGTGACAGTTGTGTAGTTGATAAAGAGATTT	480
Solanum lycopersicum_FLA456-1	GAAAGTAAAATGTAATAGCAGTAGTGGTAGTGACAGTTGTGTAGTTGATAAAGAGATTT	480
Solanum lycopersicum_FLA456-2	GAAAGTAAAATGTAATAGCAGTAGTGGTAGTGACAGTTGTGTAGTTGATAAAGAGATTT	480
Solanum lycopersicum_FLA456-3	GAAAGTAAAATGTAATAGCAGTAGTGGTAGTGACAGTTGTGTAGTTGATAAAGAGATTT	480
Solanum pimpinellifolium_WVA-1	GAAAGTAAAATGTAATAGCAGTAGTGGTAGTGACAGTTGTGTAGTTGATAAAGAGATTT	480
Solanum pimpinellifolium_WVA-2	GAAAGTAAAATGTAATAGCAGTAGTGGTAGTGACAGTTGTGTAGTTGATAAAGAGATTT	480
Solanum pimpinellifolium_WVA-3	GAAAGTAAAATGTAATAGCAGTAGTGGTAGTGACAGTTGTGTAGTTGATAAAGAGATTT	480

Solanum_lycopersicum_Ilocos_Red-1	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540
Solanum_lycopersicum_Ilocos_Red-2	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540
Solanum_lycopersicum_Ilocos_Red-3	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540
Solanum_lycopersicum_H7996-1	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540
Solanum_lycopersicum_H7996-2	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540
Solanum_lycopersicum_H7996-3	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540
Solanum_lycopersicum_Super_Apollo-1	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540
Solanum_lycopersicum_Super_Apollo-2	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540
Solanum_lycopersicum_Super_Apollo-3	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540
Solanum_lycopersicum_FLA456-1	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540
Solanum_lycopersicum_FLA456-2	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540
Solanum_lycopersicum_FLA456-3	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540
Solanum_pimpinellifolium_WVA-1	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540
Solanum_pimpinellifolium_WVA-2	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540
Solanum_pimpinellifolium_WVA-3	TGCTGATGAAGAAGATTATATAAAAAGCCGGTGGTTCGCAACTTGTATTTGTTCAAATGCA	540

Solanum_lycopersicum_Ilocos_Red-1	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600
Solanum_lycopersicum_Ilocos_Red-2	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600
Solanum_lycopersicum_Ilocos_Red-3	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600
Solanum_lycopersicum_H7996-1	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600
Solanum_lycopersicum_H7996-2	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600
Solanum_lycopersicum_H7996-3	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600
Solanum_lycopersicum_Super_Apollo-1	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600
Solanum_lycopersicum_Super_Apollo-2	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600
Solanum_lycopersicum_Super_Apollo-3	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600
Solanum_lycopersicum_FLA456-1	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600
Solanum_lycopersicum_FLA456-2	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600
Solanum_lycopersicum_FLA456-3	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600
Solanum_pimpinellifolium_WVA-1	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600
Solanum_pimpinellifolium_WVA-2	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600
Solanum_pimpinellifolium_WVA-3	GCAGAAAAAGATATGGATCAGCAGTCTAAGCTTCTGATGAGGTGATTGTAATAAATTT	600

Solanum_lycopersicum_Ilocos_Red-1	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660
Solanum_lycopersicum_Ilocos_Red-2	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660
Solanum_lycopersicum_Ilocos_Red-3	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660
Solanum_lycopersicum_H7996-1	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660
Solanum_lycopersicum_H7996-2	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660
Solanum_lycopersicum_H7996-3	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660
Solanum_lycopersicum_Super_Apollo-1	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660
Solanum_lycopersicum_Super_Apollo-2	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660
Solanum_lycopersicum_Super_Apollo-3	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660
Solanum_lycopersicum_FLA456-1	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660
Solanum_lycopersicum_FLA456-2	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660
Solanum_lycopersicum_FLA456-3	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660
Solanum_pimpinellifolium_WVA-1	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660
Solanum_pimpinellifolium_WVA-2	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660
Solanum_pimpinellifolium_WVA-3	GAAGTTATATAGTAGTCGTTTGACTCAGTTCACCGAGGACATGACCTTAAACGTAGTAGA	660

Solanum_lycopersicum_Ilocos_Red-1	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720
Solanum_lycopersicum_Ilocos_Red-2	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720
Solanum_lycopersicum_Ilocos_Red-3	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720
Solanum_lycopersicum_H7996-1	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720
Solanum_lycopersicum_H7996-2	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720
Solanum_lycopersicum_H7996-3	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720
Solanum_lycopersicum_Super_Apollo-1	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720
Solanum_lycopersicum_Super_Apollo-2	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720
Solanum_lycopersicum_Super_Apollo-3	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720
Solanum_lycopersicum_FLA456-1	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720
Solanum_lycopersicum_FLA456-2	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720
Solanum_lycopersicum_FLA456-3	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720
Solanum_pimpinellifolium_WVA-1	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720
Solanum_pimpinellifolium_WVA-2	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720
Solanum_pimpinellifolium_WVA-3	ATTCTTATCCTTTTAGTAGTATTATTTAGTTATTGTGTAGTTTCTTATTCCTTTGATGTGT	720

Solanum_lycopersicum_FLA456-3	TTTTTATCTTTCTACTTTGTGTGTCGTGTCGGATTCTTCCCTTTTGGAGCAAGGGTTTGAT	1020
Solanum_pimpinellifolium_WVA-1	TTTTTATCTTTCTACTTTGTGTGTCGTGTCGGATTCTTCCCTTTTGGAGCAAGGGTTTGAT	1020
Solanum_pimpinellifolium_WVA-2	TTTTTATCTTTCTACTTTGTGTGTCGTGTCGGATTCTTCCCTTTTGGAGCAAGGGTTTGAT	1020
Solanum_pimpinellifolium_WVA-3	TTTTTATCTTTCTACTTTGTGTGTCGTGTCGGATTCTTCCCTTTTGGAGCAAGGGTTTGAT	1020

Solanum_lycopersicum_Ilocos_Red-1	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080
Solanum_lycopersicum_Ilocos_Red-2	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080
Solanum_lycopersicum_Ilocos_Red-3	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080
Solanum_lycopersicum_H7996-1	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080
Solanum_lycopersicum_H7996-2	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080
Solanum_lycopersicum_H7996-3	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080
Solanum_lycopersicum_Super_Apollo-1	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080
Solanum_lycopersicum_Super_Apollo-2	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080
Solanum_lycopersicum_Super_Apollo-3	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080
Solanum_lycopersicum_FLA456-1	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080
Solanum_lycopersicum_FLA456-2	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080
Solanum_lycopersicum_FLA456-3	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080
Solanum_pimpinellifolium_WVA-1	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080
Solanum_pimpinellifolium_WVA-2	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080
Solanum_pimpinellifolium_WVA-3	AAGCCATGTGTGTTCCATAGTTATTCAAGGAGGAAGAAGCGGAGAAAATATAGGAGAAGAT	1080

Solanum_lycopersicum_Ilocos_Red-1	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140
Solanum_lycopersicum_Ilocos_Red-2	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140
Solanum_lycopersicum_Ilocos_Red-3	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140
Solanum_lycopersicum_H7996-1	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140
Solanum_lycopersicum_H7996-2	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140
Solanum_lycopersicum_H7996-3	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140
Solanum_lycopersicum_Super_Apollo-1	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140
Solanum_lycopersicum_Super_Apollo-2	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140
Solanum_lycopersicum_Super_Apollo-3	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140
Solanum_lycopersicum_FLA456-1	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140
Solanum_lycopersicum_FLA456-2	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140
Solanum_lycopersicum_FLA456-3	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140
Solanum_pimpinellifolium_WVA-1	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140
Solanum_pimpinellifolium_WVA-2	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140
Solanum_pimpinellifolium_WVA-3	AAATCAGTTTCTTCCATTTTTAGGAGTTATTTGGAAGTTCCTTATGGTTACTAGAAGTT	1140

Solanum_lycopersicum_Ilocos_Red-1	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200
Solanum_lycopersicum_Ilocos_Red-2	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200
Solanum_lycopersicum_Ilocos_Red-3	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200
Solanum_lycopersicum_H7996-1	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200
Solanum_lycopersicum_H7996-2	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200
Solanum_lycopersicum_H7996-3	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200
Solanum_lycopersicum_Super_Apollo-1	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200
Solanum_lycopersicum_Super_Apollo-2	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200
Solanum_lycopersicum_Super_Apollo-3	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200
Solanum_lycopersicum_FLA456-1	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200
Solanum_lycopersicum_FLA456-2	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200
Solanum_lycopersicum_FLA456-3	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200
Solanum_pimpinellifolium_WVA-1	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200
Solanum_pimpinellifolium_WVA-2	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200
Solanum_pimpinellifolium_WVA-3	ATGTGCTTTTATCCTAACTGGTTATGAAGTAGGTATATTTAGTTATTATATAAATAATAG	1200

Solanum_lycopersicum_Ilocos_Red-1	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245
Solanum_lycopersicum_Ilocos_Red-2	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245
Solanum_lycopersicum_Ilocos_Red-3	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245
Solanum_lycopersicum_H7996-1	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245
Solanum_lycopersicum_H7996-2	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245
Solanum_lycopersicum_H7996-3	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245
Solanum_lycopersicum_Super_Apollo-1	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245
Solanum_lycopersicum_Super_Apollo-2	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245
Solanum_lycopersicum_Super_Apollo-3	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245
Solanum_lycopersicum_FLA456-1	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245
Solanum_lycopersicum_FLA456-2	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245
Solanum_lycopersicum_FLA456-3	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245
Solanum_pimpinellifolium_WVA-1	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245
Solanum_pimpinellifolium_WVA-2	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245
Solanum_pimpinellifolium_WVA-3	ATCTAGTAATGTAGCTATGTTGAAAATTAATCTTTTATATGCAA	1245

Appendix Figure III. Alignment of Lycopene epsilon-cyclase gene sequences isolated from the selected tomato accessions. The locations of the four SNPs found along the 1245bp sequence covering the 5' UTR and Exon 1 of the gene are highlighted in yellow.