



Genome-Wide Identification and Analysis of Lycopene Gene Family in Tomato (*Solanum lycopersicum* L.)

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Abstract: Tomato (*Solanum lycopersicum* L.) is an important vegetable crop and a major dietary source of lycopene, a naturally occurring carotenoid responsible for the characteristic red colour of ripe tomato fruits and recognized for its potent antioxidant properties. Despite the nutritional and biological importance of lycopene, there is limited information on the genomic organization and evolutionary characteristics of the lycopene-related gene family in tomato. In this study, an in-silico genome-wide analysis was conducted to identify and characterize the SILYC gene family in *S. lycopersicum*. Lycopene-related genes were identified through genome-wide sequence analysis using *Arabidopsis thaliana* LYC gene sequences as queries, followed by verification of the identified proteins using InterPro. Chromosomal distribution, gene structure, subcellular localization, phylogenetic relationships, and conserved protein motifs were subsequently analyzed using appropriate bioinformatics tools. A total of four non-redundant SILYC genes were identified and mapped to chromosomes 4, 6, 10, and 12 of the tomato genome. Subcellular localization analysis predicted that three SILYC proteins were localized in the chloroplast, while one was predicted to be localized in the nucleus. Gene structure analysis revealed variation in exon-intron organization, with two of the identified genes being intronless. Phylogenetic analysis grouped the identified SILYC proteins into three major groups in relation to LYC proteins from *Arabidopsis thaliana*. In addition, three conserved motifs were identified across the SILYC protein sequences, indicating functional conservation within the gene family. These findings provide useful information on the genomic organization, evolutionary relationships, and structural characteristics of the SILYC gene family and may support future molecular studies, genetic improvement, and breeding strategies aimed at enhancing lycopene content and nutritional quality in tomato.

Keywords: Tomato; Lycopene; SILYC gene; Biosynthesis; Genome evolution

1. Introduction

Tomato (*Solanum lycopersicum* L.), a member of the Solanaceae family, is the second most significant fruit or vegetable crop globally after potato (*Solanum tuberosum* L.). According to FAO (2019), around 182.3 million tons of tomato fruits are harvested annually across 4.85 million hectares of land. Asia is the leading producer, contributing 61.1% of global tomato output, followed by Europe (13.5%), the Americas (12.4%) and Africa (11.8%). Tomato serves as one of the most extensively studied model plants, particularly valuable for scientific research focused on fruit development and quality traits (Karlovė *et al.*, 2014; Kim *et al.*, 2018; Liu *et al.*, 2018). Both cultivated tomato and its wild relatives are diploid species, possessing 12 chromosomes ($2n=2x=24$), which were first identified by Barton in 1950. These chromosomes are structurally divided into pericentric

heterochromatin and distal euchromatin regions. The tomato genome is relatively compact, with an estimated size of about 950 mega bases (Mb). Repetitive sequences, which constitute roughly 30% of the genome, are predominantly found in the heterochromatic regions (Van der Hoeven *et al.*, 2002). The genome encodes approximately 35,000 genes, most of which are densely arranged in the euchromatic regions.

Lycopene as a phytonutrient and powerful antioxidant, is a naturally occurring carotenoid responsible for the vibrant red colour in tomato, watermelon and pink grapefruit (Alnökkari, 2023). Tomato-derived lycopene has been shown to possess the strongest antioxidant capacity and singlet oxygen-quenching potential among all dietary carotenoids (Imran *et al.*, 2020; Przybylska,

2020). Given its health benefits, regular consumption of lycopene through tomatoes is considered a promising strategy for the prevention of chronic diseases such as cancer, heart disease, and diabetes (Alda *et al.*, 2009; Camera *et al.*, 2013). Furthermore, lycopene is known to enhance intercellular communication, influence hormonal and immune responses, and regulate various metabolic pathways (Amer *et al.*, 2020). Lycopene concentration in tomatoes varies by species and typically increases as the fruit matures and ripens (Yoo *et al.*, 2017). The noticeable shift in colour during the ripening process is primarily due to the breakdown of chlorophyll and the subsequent accumulation of lycopene, and acyclic carotenoid in both the fruit skin and pulp (Seymour *et al.*, 2013; Borghesi *et al.*, 2016; D'Ambrosio *et al.*, 2018). Lycopene is produced via carotenoids biosynthesis pathway which begins with the production of phytoene, a colourless compound synthesized from geranylgeranyl diphosphate through the enzymatic action of phytoene synthase (PSY). Subsequently, three key enzymes' phytoene desaturase (PDS), ζ -carotene desaturase (ZDS), and carotenoid isomerase (CRTISO) work sequentially to convert phytoene into lycopene via intermediates such as phytofluene and ζ -carotene. After the formation of lycopene, the carotenoid pathway branches into two directions, producing α - and β -carotenoids through the catalytic actions of lycopene cyclase and lycopene β -cyclase enzymes (Cazzonelli *et al.*, 2010; Shi *et al.*, 2014).

Despite the importance of lycopene in determining tomato fruit and nutritional value, there is a limited understanding of the lycopene gene family in tomato. This research provides an in-depth analysis of the lycopene gene family in tomato. This can contribute by providing insight into plant breeding and genetic by supporting genomics-guided selection and the identification of key genes for trait improvement.

2. Materials and Methods

Identification and Characterization of Lycopene Gene in *Solanum lycopersicum*

Lycopene related genes were identified using the *S. lycopersicum* whole genome released by Ensembl plant (<http://plant.ensembl.org>) via blast search, using the Arabidopsis gene sequence as queries. Arabidopsis gene sequences were obtained from the TAIR10 genome assembly released by NCBI (<http://www.ncbi.nlm.nih.gov>). All the protein sequences of *S. lycopersicum* for LYC gene were further verified using InterPro 102.0: <http://www.ebi.ac.uk/interpro> (Paysan *et al.*, 2022). Additionally, all LYC protein sequence of *Arabidopsis thaliana* was retrieved from plant Ensembl and verified using InterPro.

Chromosomal location, Gene structure and Subcellular localization

Chromosomal locations of the identified SILYC genes were displayed using the mapgene2chrom 2.1 software (<http://mg2c.iask.in>). Tandem duplicate genes were defined as adjacent homologous gene on a single chromosome with no more than one intervening gene (Zhao *et al.*, 2014). For gene structure, exon-intron structure of SILYC genes were examined by comparing CDS sequence and corresponding genomic sequence using online Gene Structure Display Server-GSDS 2.0: <http://gsds.cbi.pku.edu.cn> (Hu *et al.*, 2015). The subcellular localization of SILYC protein was predicted using plant subcellular localization integrative predictor (PSI predictor) <http://bis.zju.edu.cn/psi> according to method of Liu *et al.* (2013) using P-value of <0.05.

Phylogenetic Analysis and Identification of Conserved Motifs

The amino acid sequence of SILYC were imported into MEGAX and multiple sequence alignment was performed using ClustalW Omega with a gap and gap extension penalties of 10 and 0.1 respectively (Tamura *et al.*, 2011). The aligned sequences were used to construct an unrooted phylogenetic tree based on the neighbor-joining (NJ) method using bootstrap values of 1,000 replicates (Saitou and Nei, 1987) and the tree was displayed using Interactive Tree of Life- iTOL: <http://itol.embl.de/index.shtml> (Letunic and Bork, 2011). To deduce the evolutionary relationship of LYC proteins among Tomato species (*Solanum lycopersicum*) and *Arabidopsis thaliana*, additional LYC proteins were downloaded from plant Ensembl database as described above and used to construct an unrooted phylogenetic tree (Jin *et al.*, 2017). Evolutionary analyses were conducted in MEGA X and the number of bootstrap replicates will be 1,000. For conserved motifs identification, multiple EM for motif elicitation (MEME) suites- <http://meme-suite.org/> was used (Bailey, 2009). To identify motifs within the SILYC subfamily, with the following parameters: any number of repetitions, a maximum of 10 mismatches, and an optimum motif size of 6–70 amino acid residues.

3. Results

Identification and Characterization of Lycopene Gene in *Solanum lycopersicum*

Arabidopsis gene and Ensembl plant (<http://plant.ensembl.org>) were used to comprehensively identify the SILYC gene. In total we identified 4 non-redundant LYC gene member which were further named according to the name of their SILYC orthologs.

Chromosomal location, Gene structure and Subcellular localization

To examine the genomic distribution of the identified SILYC gene, their approximate location on chromosome were analyzed. All the identified SILYC gene were mapped on the chromosome of *S. lycopersicum* named as 4,6,10 and 12 (Figure 1). To investigate the evolution of

SILYC gene the intron-exon architecture was analyzed by comparing the CDS sequence and genomic sequence of each SILYC gene (Figure 2). Among the 4 of the SILYC gene, 1 having 11 exon and 10 introns (1, 25%), 1 with 6 exon and 5 introns (1, 25%) while the remaining 2 are intron less with only exon (2, 50%). 1 of

SILYC gene having upstream\downstream. 2 are up to 1.5kb, 1 up to 6kb and 1 $\geq$ 6kb. The subcellular localization analysis showed 3 SILYC gene were localized in the chloroplast of the cell while 1SILYC gene was in the nucleus.

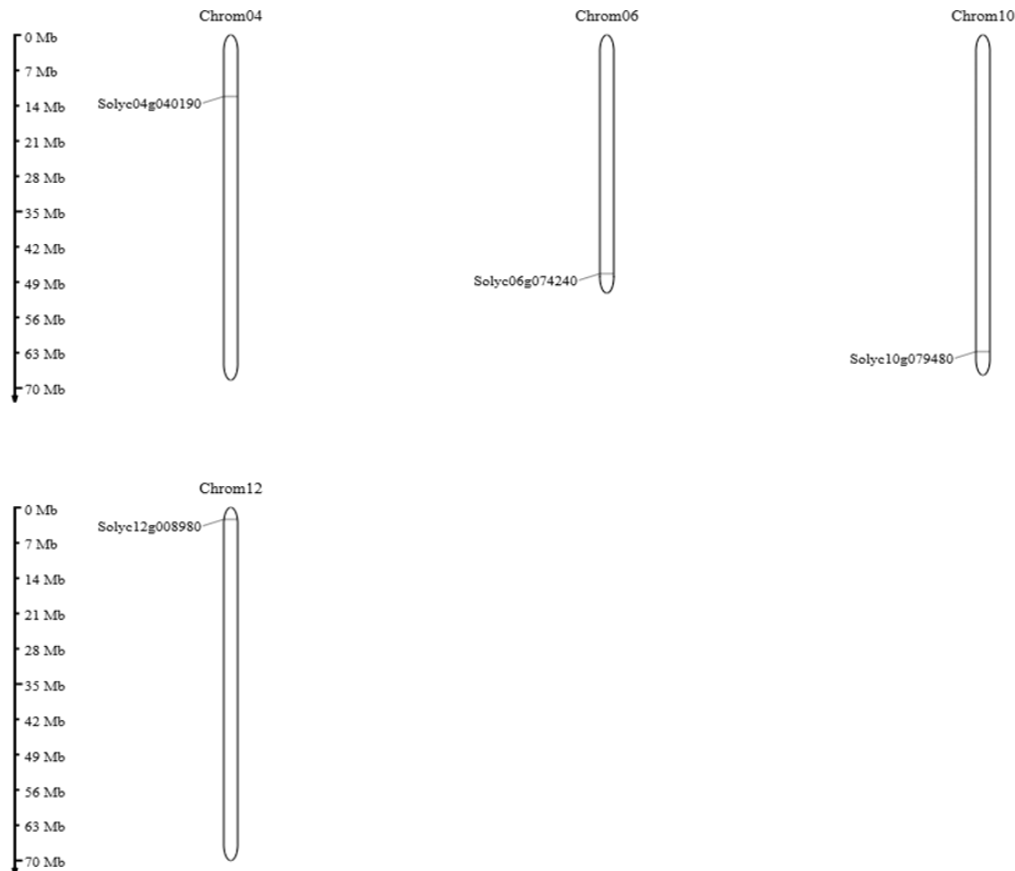


Figure 1: Chromosomal location of identified of SILYC gene. 4 SILYC gene were mapped on chromosome (4, 6, 10 and 12). A scale on the left shows the chromosome length (Mb)

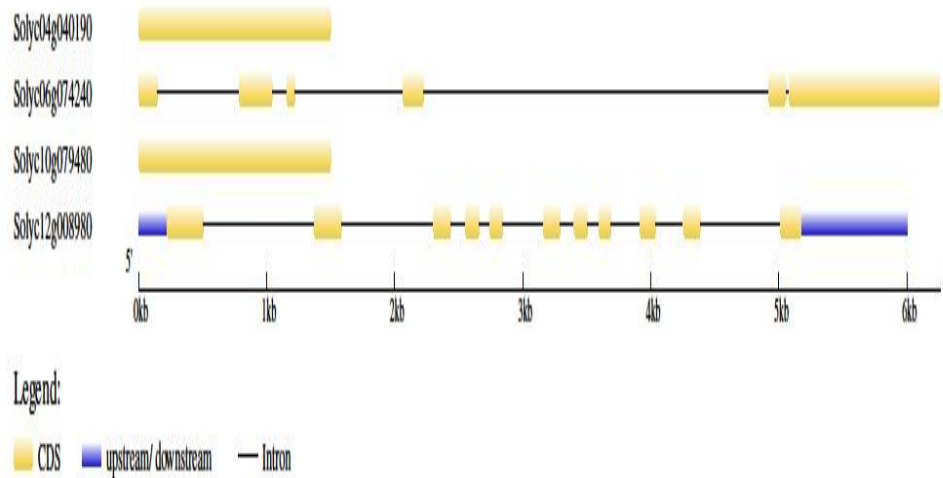


Figure 2: Exon-intron structure of each *SILYC* gene is proportionally displayed in terms of the scale at the bottom. Golden bars represent exons, black lines represent introns and blue bars represent upstream/downstream. Most of the *SILYC* genes are intronless consisting only of exons and are <1,500 bp in length.

Phylogenetic Analysis and Identification of Conserved Motifs

To explore the phylogenetic relationship of identified LYC protein in *S. lycopersicum*, a phylogenetic tree of *SILYC* and *Arabidopsis* LYC protein was constructed using the neighbor-joining method. According to the phylogenetic result *SILYC* protein was grouped in 3, group 1 consist of 2 *SILY* protein and 1 *Arabidopsis* LYC protein, group 2 consist of 1 *SILYC* protein only and group 3 consist of 1 *SILYC* protein and 1 *Arabidopsis* LYC protein. This revealed that the LYC protein *S. lycopersicum* is higher than that of *Arabidopsis*. (Figure 3). Motif Conserved analysis, a total of 3 conserved motif were identified in the *SILYC* protei using meme software. The 3 motifs occur in all the identified *SILYC* protein (Figure 4).

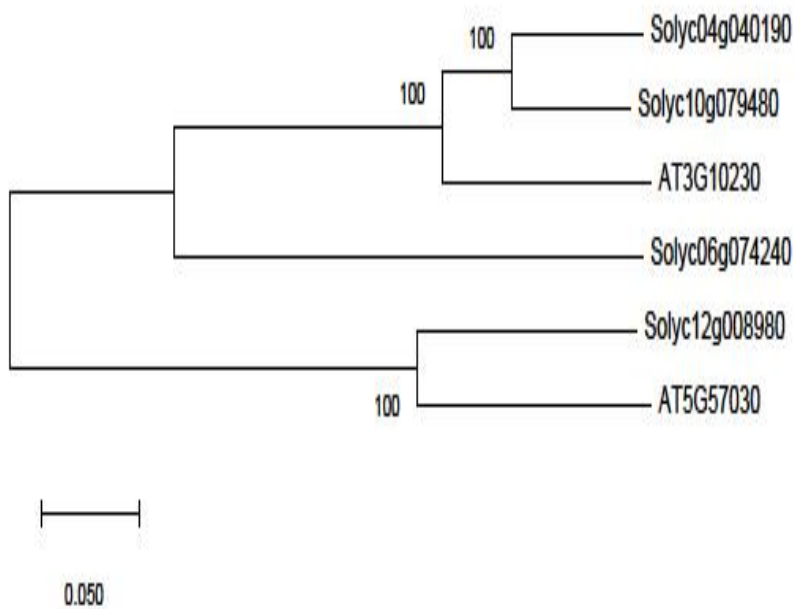


Figure 3: Phylogenetic analysis of LYC proteins between *Solanum lycopersicum* and *Arabidopsis thaliana*. A total of 6 (4 SILYC and 2 AtLYC) conserved protein domains were aligned using ClustalW, and the phylogenetic tree was constructed by MEGA X using the neighbour-joining method with 1,000 bootstrap replicates.

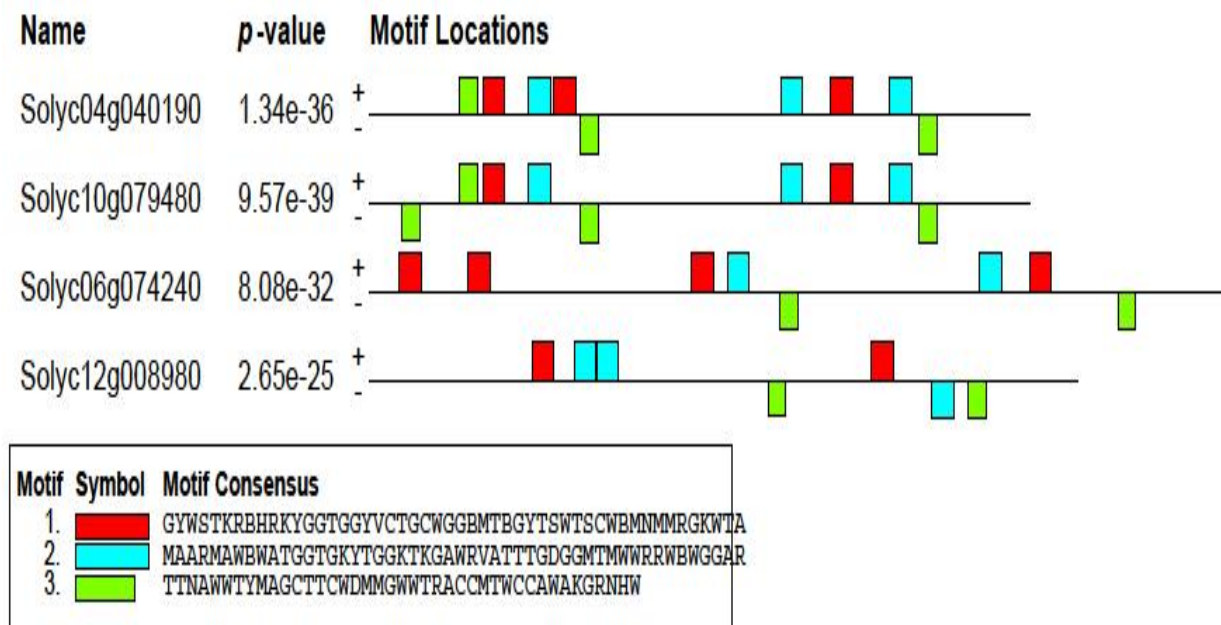


Figure 4: Conserved motif of SILYC protein. Different coloured boxes represent different conserved motifs and their consensus sequences were shown below.

4. Discussion

In this study, the total number of LYC genes in *S. lycopersicum* is higher than that of *Arabidopsis thaliana* (2) and lower than the Carotenoid metabolism related gene in grapevine fruit {54} (Xiangpeng, 2017) and cabbage {67} (Wenxue, 2025) and Among the studied species, the genome size of *S. lycopersicum* (950mb) is larger than that of *Arabidopsis thaliana* (135 Mb), grapevine fruit (500 Mb) and cabbage (530 Mb) the diversity in genome size of plants may influence the number of carotenoid gene in plant species and a larger genome may allow for more genetic diversity and variation in carotenoid biosynthesis. Lycopene can subsequently undergo cyclization to form other carotenoids; α and β carotenoid through the action of two lycopene cyclase and lycopene β -cyclase (Cazzonelli *et al.*, 2010; Shi *et al.*, 2014). Carotenoids participate in a wide range of physiological processes, including plant growth, development, and responses to environmental stimuli. Besides their functions as pigments and nutrients, carotenoids are also the precursors of many important volatile flavor compounds in plants, conferring the sensory attribute that can be detected by consumers (Vogel *et al.*, 2010).

To examine the genomic distribution of the identified SILYC gene, their approximate location on chromosome were analyzed. All the identified 4 SILYC gene were mapped on the chromosome *S. lycopersicum* number 4,6,10 and 12. 46 out of the 62-carotenoid gene in

cabbage are located on the chromosome C01-C09, respectively while 16 on the scaffold. In our study, the intron-exon architecture of the 4 LYC gene revealed that 1 having 11 exons and 10 introns (1,25%), 1 with 6 exons and 5 introns (1,25%) while the remaining are intron less, (2, 50%) having only exons. The existence of introns in a gene leads to alternative splicing of the gene product. The process of removing immature mRNA by splicing occurs in a majority of eukaryotic protein-coding genes, producing variant transcripts of the same gene (Filichkin *et al.*, 2015). Alternative splicing affects transcript export, protein translation, and degradation (Reddy *et al.*, 2013). Though alternative splicing in plants is changeable during the growth, developmental process, and environmental stresses (Staiger and Brown, 2013). Motif analysis among SILYC protein sequence indicated a conserved array of the motif across all identified member of LYC subfamily.

The phylogenetic analysis revealed the presence of 4 LYC protein *S. lycopersicum*, divided into three major groups. Group 1 included 2 SILYC protein, while group 2 and 3 contained 1 each. *Arabidopsis thaliana* possessed only one LYC protein in group 1 and 3. This disparity in the number of LYC homologs between tomato and Arabidopsis suggests a possible gene duplication event in tomato leading to the expansion of LYC gene family. Evolutionary changes have helped plants adapt to both abiotic and biotic stresses by

controlling a network of certain genes through the modulation of specific transcription factors (Nan and Goe, 2019). The subcellular location tool predicted 3 SILYC are chloroplast protein while the remaining 1 is a nucleus protein. Carotenoids are formed and sequestered at very high levels in plastids, mainly chloroplasts and chromoplasts (Egea *et al.*, 2010).

5. Conclusion

Genomic analysis identified 4 non-redundant lycopene gene distributed across chromosomes 4, 6, 10 and 12. Gene structure analysis revealed variation in exon-intron architecture with some gene being intron less. Subcellular localization indicates that most SILYC gene are chloroplast localized, aligned with their role in pigment biosynthesis. SILYC gene were grouped in to 3 based on phylogenetic analysis. Conserved motif in SILYC protein shown functional conservation with in the gene family. The finding provides valuable insight for breeding programs aimed at enhancing lycopene content and improving nutritional quality.

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