JNRID || ISSN 2984-8687 || © April 2024, Volume 2, Issue 4

RECENT BREEDING APPROACHES FOR IMPROVEMENT OF QUALITY TRAITS IN SOLANACEOUS VEGETABLE CROPS

[1]Ajay S. M., [2]S. G. Angadi and [3]R. M. Hosamani

[1]Ph. D Scholar, [2]Professor of Horticulture, [3]Professor of Horticulture
Department of Horticulture
College of Agriculture, University of Agricultural Sciences, Dharwad, India.

Abstract - Vegetable crops are rich in dietary micronutrients, antioxidants and functional phytochemicals. Solanaceous vegetables are key sources of health protective dietary constituents such as minerals, vitamins and antioxidants in human diet. However, some of the elements are of public health concern such as β-carotene, iron, calcium and folic acid which attracts breeder's interest to improve their content in food sources including vegetable crops so that their dietary intake could be increased and their deficiency problems reduced. The attempts through conventional breeding methods have been slow and inadequate, hence new developments in molecular and biochemical diagnostics tools and techniques showed promise in improving the nutraceutical contents and other quality traits. Molecular markers, next generation sequencing, RNA interference (RNAi) and genetic engineering have great promise in reducing time and cost involved in quality breeding. The present review summarises attempts and potential of improving the nutraceuticals and quality traits in vegetable crops. Among the different reviewers they observed different results; the transgenic approach has achieved high levels of anthocyanin content throughout the fruit. In relatively remote rural areas where traditional foods are predominant in local diets and there is problem of food shortage during the off-season, the vegetable varieties with improved nutritional values and better storage-cum-transport life can serve much better. Advance tools and techniques of biochemical analysis such as high-performance liquid chromatography, gas chromatography-mass spectrometry and inductively coupled plasma mass spectrometry for rapid detection of compounds in advance stage breeding materials.

Keywords - Vegetable crops, Nutraceutical, Breeding, Molecular markers, Anthocyanin.

I. INTRODUCTION

Solanaceous vegetable crops, which belong to the Solanaceae family, play a vital role in global agriculture and human nutrition (Sakamoto *et al*., 2017). This diverse group includes important crops such as tomatoes (*Solanum lycopersicum*), potatoes (*Solanum tuberosum*), peppers (*Capsicum spp*.) and eggplants (*Solanum melongena*). These crops are not only staple foods in many diets but also contribute significantly to the economic livelihoods of farmers and the agri-food industry. Over the years, there has been a growing demand for solanaceous vegetables with improved quality traits to meet consumer preferences and nutritional requirements (Wang *et al*., 2014). Quality traits encompass a wide range of characteristics, including taste, texture, nutritional content, disease resistance, and shelf life. To address these demands, recent breeding approaches have focused on integrating advanced techniques to expedite the development of improved varieties.

Traditional breeding methods have been effective in enhancing certain traits, but the pace of improvement can be slow. Recent advances in molecular biology, genomics, and biotechnology have opened up new possibilities for accelerating the breeding process and achieving precise modifications in target traits (Jeong *et al*., 2015). This review explores the recent breeding approaches employed for the improvement of quality traits in solanaceous vegetable crops. It delves into the integration of traditional breeding methods with modern tools, such as molecular markers, genetic engineering, and genomic selection, to enhance traits like flavor, nutritional content, disease resistance, and post-harvest attributes. The synergistic application of these approaches not only expedites the breeding process but also enables the development of varieties that meet the diverse needs of consumers while addressing challenges such as climate change and emerging diseases (Xu *et al*., 2015). The following sections will provide an in-depth analysis of specific breeding strategies employed in the improvement of quality traits in solanaceous vegetables, highlighting successful case studies and future prospects for sustainable and resilient crop improvement

This review article is based on Doctoral Seminar- I presented during semester-II of year 2022-2023.

Nutraceuticals in vegetables

Vegetables form an essential component of a balanced diet and provide a significant share of dietary demand of minerals and vitamins. Nutraceuticals are among the important constituents of quality attributes in vegetable crops. Nutraceuticals and bioactive play a key role in protection against cancer, heart disease and stroke (Behera and Singh, 2019). The β-carotene is important for healthy skin and membranes, immune system, and good for eye health; lycopene from tomato, watermelon, carrot and red peppers fights against prostate cancer; ascorbic acid rich green peppers, broccoli, green leafy vegetables, cabbage and tomatoes is helpful in growth, development and repair of all body tissues and absorption of iron from foods. The momordicin and charantin from bitter gourd have anti-diabetic properties and help in blood purification. Most vegetables are naturally low in fat and calories while none have cholesterol, hence, good for heart. Dietary fibre reduces blood cholesterol levels, lowers risk of heart disease and improves bowel functioning.

Study of genetic behaviours, help the breeder in formulating effective breeding plan for its improvement. Wild relatives of a crop including its primitive forms, related weedy species and other species in the same genus, which are not under cultivation, serve as potential sources of genes

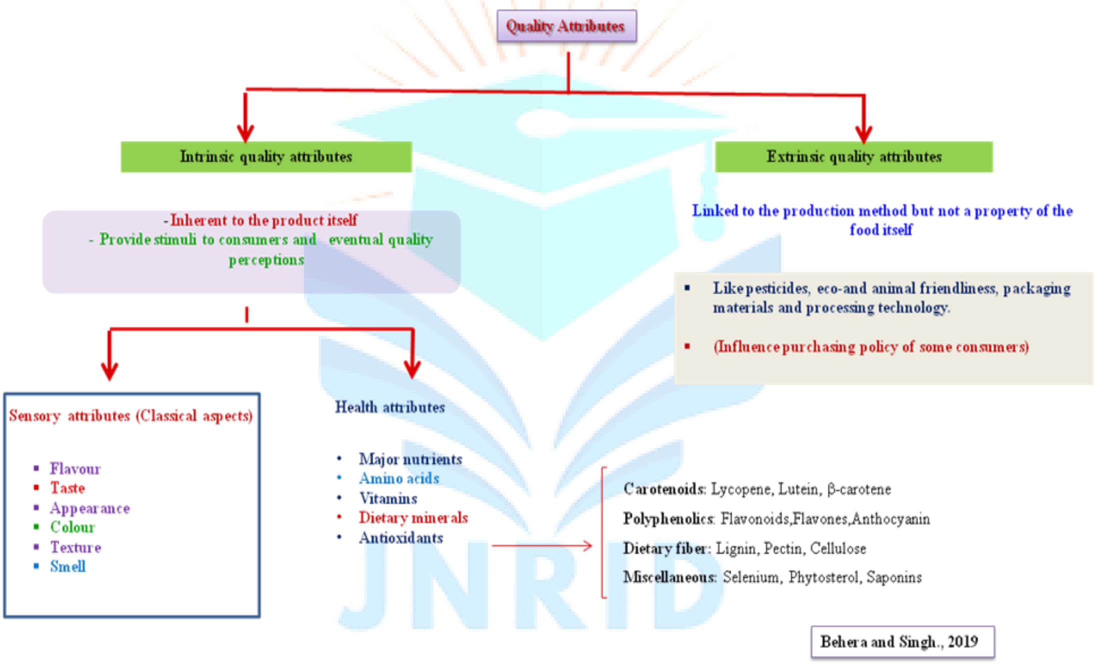

Fig. 1. Classification of quality traits in vegetable crops

Table 1. Solanaceous vegetables

Crop	Scientific Name	Nutritional compounds	Breeding behavior
Tomato (2n=2x=24)	*Solanum lycopersicum* L.	Lycopene beta- carotene	Self pollinated
Brinjal (2n=2x=24)	*Solanum melongena* L.	Nasunin cholorogenic acid	Often-cross pollinated
Chilli (2n=2x=24)	*Capsicum annum* L.	Capsaicinoids carotenoids	Often-cross pollinated
Potato (2n=4x=48)	*Solanum tuberosum* L.	Carotenoids Flavonoids caffeic acid	Self pollinated

Gene action refers to way in which certain genes exert their effects on plant system. Additive gene action effects occurs when two or more genes source a single contribution to the final phenotype or when allele of single gene combine so that their combined effects equal to sum of their individual effects (Xindi *et al*., 2018). Non additive gene action refers to a condition wherein one allele is expressed stronger than another allele. Gene action of some quality traits is listed in Table 2.

Table 2. Gene action of important quality traits

Crop	Character	Gene action
Tomato	Fruit Weight	Non-additive
	TSS, Vit C, Carotenoids, Lycopene	Non-additive
	Pericarp thickness	Additive
	Locule number	Additive
Brinjal	Fruit Weight	Additive
	Fruit size index	Additive
	Fruit length	Additive
	Fruit width	Additive
Chilli	Fruit Weight	Additive
	Pungency	Additive
	Fruit length	Additive

Genetics is the study of heredity and variation of inherited characteristics. Genetics of some quality traits of solanaceous vegetable are give in the Table 3 (Fischer *et al*., 2018).

Table 3. Genetics of quality traits

Crops	Traits	Gene	Features
Tomato	Lycopene	Og^c, *hp*	Red pigment
	Vitamin C	*Vtc*	High Vit C
	Shelf life	*Rin, nor, Nr*	Non-ripening
Chilli	Capsaicin	*Pun, Cap*	High pungency
	Capsanthin	*C*	High capsanthin
Brinjal	Anthocyanin	*fap*	Anthocyanin accumulation
	Fruit stripe	*fst*	Striped fruits
Potato	Zeaxanthin	*Chy*	Zeaxanthin accumulation
	Anthocyanin	*Stan*	Anthocyanin accumulation

Advanced breeding methods:

Advance quality breeding needs a combination of genetic source, modern genomic tools and biochemical diagnostic techniques. The breeding methods includes,

- Mutation breeding
- Molecular breeding
- SNP’s discovery
- RNA interference
- Targeted genome editing

Mutation Breeding

The genetic improvement of crop plants for various economic traits through the use of induced mutation (mutation that are induced by mutagenic agents: gamma rays, E.M.S., etc.) is referred as mutation breeding (Swathy *et al*., 2016).

Important steps to know before mutation breeding are,

1. Selection of variety for mutation breeding: Generally, variety selected for mutagen treatment should be best commercial variety of the crop.

2. Part of the plant to be treated: In sexually propagated plant, seeds are most commonly used for mutagen treatment. Pollen grains may be used in some cases.

3. Dose of the mutagen: Dose of the mutagen should be such that it induces maximum frequency of mutations, while is causes minimum killing.

4. Mutagen treatment: Seeds which are to be treated with chemical mutagen are first soaked in water for few hours. This initiates some metabolic activities and then seeds are treated with desired dose of chosen mutagen.

Molecular Breeding

- Molecular markers enable unambiguous identification of lines/individuals in segregating population for handling of quality traits.
- MAS: Indirect selection for a phenotype based on banding pattern of linked molecular markers.
- Effective in introgression of desirable genes from wild into cultivated genotypes.
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Table 4. Molecular breeding for quality traits

Crop	Marker	Trait	Reference
Tomato	SCAR	high-lycopene	Lixia *et al.*, 2011
Chilli	CAPS	High Pungency in *C. Chinense*	Tanaka *et al.*, 2016
Potato	SSR	Cold induced sweetness	Fischer *et al.*, 2013

Single Nucleotide Polymorphism

- It is the most common form of DNA sequence variation between alleles, in several plant species.

- SNP’s have become choice markers due to their abundance, stability, amenability to automation, and cost-effectiveness.

- The selection of SNP’s enables the selection of desired lines in large-scale populations. SNPs can also be used to discover new genes and their functions by affecting gene expression. The advantages of SNP is that they are abundant in number and SNP detection is more rapid because it is based on oligonucleotide hybridization analysis with one disadvantage being there is high possibility that a SNP does not display any variability in family that is being studied.

RNA Interference (RNAi)

- The RNA silencing is a gene regulatory mechanism that limits the transcript level by suppressing transcription. This approach was effectively used to alter the gene expressions for improving quality traits.

- The first transgenic cultivar, ‘Flavr-Savr’ variety of tomato developed in 1994 by calgene company.

- The development of transgenic plants has continued mainly with the introgression of genes-disease resistance and to fruit quality.

Genome Editing

- Genome editing refers to alteration of DNA sequences to modify gene function for obtaining desirable traits.
- Genome editing can effectively induce targeted mutations in plant genomes at precise location, reduces non-specific off-target cleavage, highly efficient and site-specific performance observed. Final products- Identical to the mutants obtained by 'conventional' mutagenesis.

Marker-assisted selection has emerged as a powerful tool in solanaceous vegetable breeding to enhance the efficiency of trait selection. According to the Li and his co-workers promoting the biosynthesis of Lycopene and to knockdown some genes associated with the conversion of Lycopene to α and β-carotene. Carotenoid biosynthesis depends on isopentenyl diphosphate (IPP) and its isomer DMAPP. In plastids, four molecules of IPP were condensed to a molecule of GGPP. Then, two molecules of GGPP can be catalyzed by phytoene synthase 1 (PSY1) to form a molecule of colourless 15-cis-phytoene, which are head-to head condensed, leading to the generation of z-carotene and pink prolycopene.

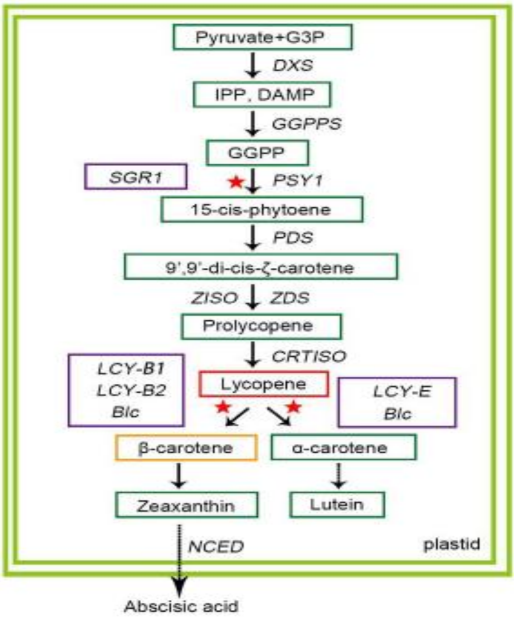


Fig. 2. Carotenoid Metabolic Pathway

Table 5. Efficient Multiplex CRISPR/Cas9-Mediated Targeted Mutagenesis in Tomato Plants

Target	No. of plants examined	No. of plants with mutations	Mutation rate %
T1	24	10	41.67
T2		23	95.83
T3		2	8.33
T4		22	91.67
T5		0	0
T6		1	4.17

In all, 24 lines of transgenic plants were obtained, specific editing types of each target were identified and analyzed. Fortunately, the 24 transgenic lines showed different types of genomes editing at the target sites which indicated that multiplex CRISPR/Cas9 is extremely efficient in tomato fruit to generate tailor-made modifications at target sequences. The mutation rates varied widely among different target sites, from 0 to 95.83%. The editing efficiencies of targets T2 and T4, especially target T2 (95.83%), were considerably higher than those of the others. However, target T5 did not show gene editing in any of the obtained transgenic lines.

The mutation rate is the ratio of the number of mutations detected to that of the total number of plants in which mutations are detected (Table 5.).

Table 6. 24 transgenic tomato plants were classified into 5 mutant groups according to the different mutant target genes

Group	Mutants	Edited Genes	Number
Lycopene - 1	Single	SGR1	2
Lycopene - 2	Single	Blc	1
Lycopene - 3	Double	SGR1, Blc	19
Lycopene - 4	Triple	SGR1, Blc, LYC-E	1
Lycopene - 5	Quadruple	SGR1, Blc, LYC-E, LCY- B2	1

The levels of carotenoids were determined by classifying the 24 transgenic tomato plants into 5 mutant groups according to the different mutant target genes, including single, double, triple, and quadruple mutants, which were named as Lycopene-1 to 5. Representative transgenic lines were selected from each group for further analysis (Table 6.).

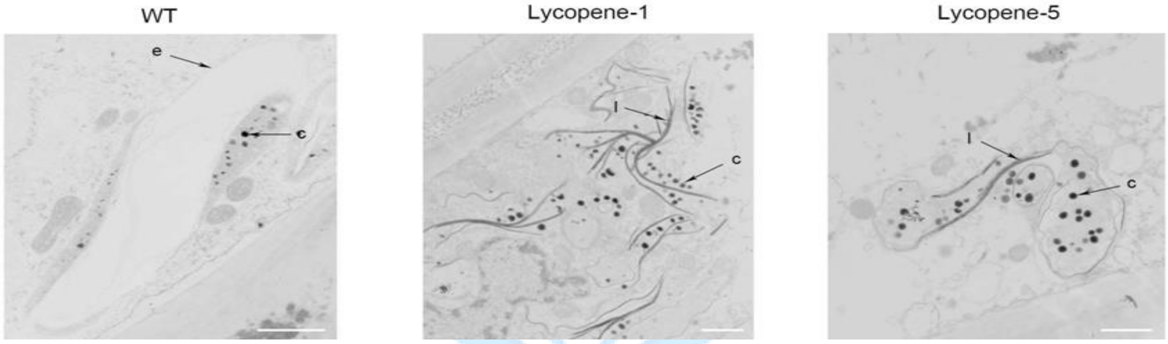


Fig. 3. Epidermal cells in tomato fruit of WT and lycopene mutants. c-carotenoid containing structures e-plastid envelope l-crystal line.

Result revealed that unlike in WT, in Lycopene-1 and Lycopene-5, the plastid numbers in the pericarp cells were significantly higher at Br+7. Further, carotenoid-containing structures (osmiophilic globules) and crystal lines were higher in the plastids of lycopene mutant fruits than in WT fruits the conversion of chloroplasts to chromoplasts might occur earlier in transgenic fruits than in WT (Fig. 3.).

Plant transformation and screening for the transgene, forty-six primary tomato transformants were generated using Agrobacterium-mediated transformation using pGAntho binary vector. PCR screening using Del, Ros1 gene-specific primers confirmed the presence of the transgene in the primary transformants and two lines were selected for further analysis (Maligeppagol *et al*, 2013).

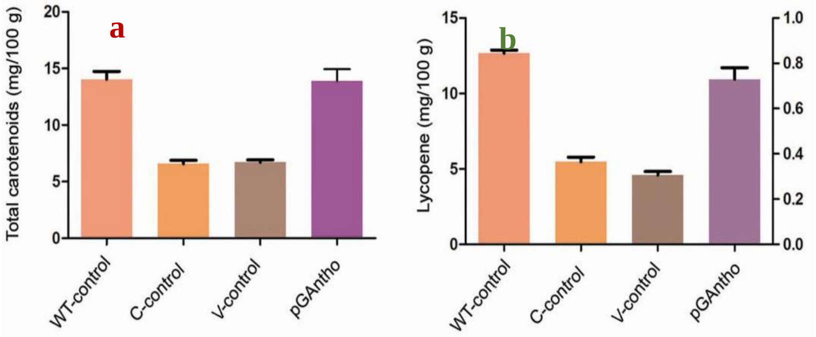


Fig. 4. Biochemical analysis of tomato fruit. Total carotenoid content; Lycopene content. WT, Wild type; C, Commercial; V, vector and pGAntho, Transgenic tomato.

The total carotenoid content of the transgenic fruits (13.89 mg/100 g) was on par with that of the WT control, while it was nearly two-fold higher than that of commercial control fruits (Figure a 4) and the lycopene content of the transgenic fruits and WT control fruits did not differ significantly and was 10.93 mg and 12.65 mg per 100 g fruit respectively (Maligeppagol *et al.*, 2015), whereas commercial control fruits had only half as much lycopene as the anthocyanin-rich fruits Figure b 4.

Gene expression analysis

Del and Ros1 expression were highly dependent on fruit developmental stages. The expression levels were lower in green stage. In breaker-stage fruits, the expression of Del and Ros1 showed 10.15- and 24.43-folds upregulation respectively. However, in red-ripe fruit, the expression levels were drastically reduced (Figure 5 a). Further, the expression of target genes of Del and Ros1, viz. chalcone isomerase (CHI) and flavanone-3-hydroxylase (F3H) were analysed across the development stages of the fruits. The expression levels of CHI and F3H were 5.69 and 6.63-fold higher respectively, in breaker stage compared to green and red-ripe stages. In addition, the expression levels of CHI and F3H were several folds higher in the transgenic fruits compared to the WT fruits (Figure 5 b).

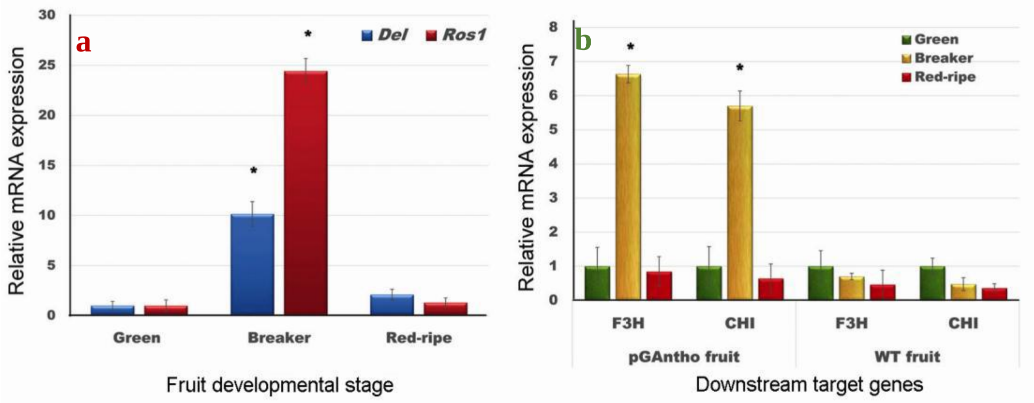


Fig.5. Expression analysis of *Delia* and *Rosea* and their targets*:* a. Relative mRNA levels of *Delia* and *Rosea* at three developmental stages of fruit. b. Relative mRNA levels of F3H and CHI induced by *Delia* and *Rosea* at three developmental stages of fruit

Anthocyanin estimation revealed higher accumulation of anthocyanins in the transgenic fruits compared to the WT, vector control and commercial control fruits. The average anthocyanin content of the transgenic fruit was 0.1 mg/g fresh weight, which was 70–100 fold higher than that of the control fruits. Lycopene and β-Carotene Contents of Mutant Fruits Were Remarkably Enhanced by CRISPR/Cas9-Mediated Gene Editing (Xindi *et al.*, 2018). HPLC analysis showed that the contents of lycopene and β-carotene in all lycopene mutants were higher than those in the WT plants (Fig. 5)

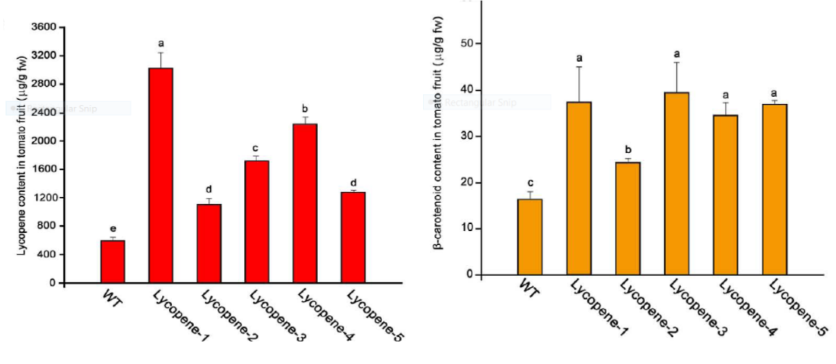
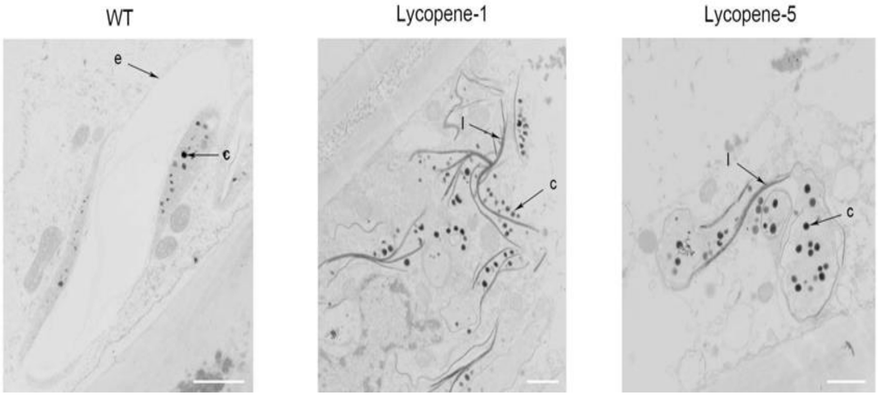


Fig. 6. Lycopene and b- carotene contents of 5 different lycopene groups along with WT by using HPLC analysis

However, mutation of the Blc gene alone (Lycopene-2) did not remarkably improve lycopene accumulation compared with that in the Lycopene-1 group (Figure 6), indicating that the effect of SGR1 gene on the regulation of lycopene content in tomato fruit was more pronounced than that of Blc gene**.** HPLC analysis showed that the contents of lycopene and β-carotene in all lycopene mutants were higher than those in the Wild Type plants. In particular, the lycopene content of group Lycopene-1, in which the *SGR1* gene was targeted alone, was the highest (5.1-fold). Carotene content of group Lycopene-3, in which the *SGR1 and blc* was targeted, was the highest.

Transmission Electron Microscope Analysis



c-carotenoid containing structures **e-**plastid envelope **l-**crystal line
Fig. 7. Epidermal cells in tomato fruit of WT and lycopene mutants

To further confirm the improvement of lycopene accumulation at the cellular level, we used TEM to observe the tomato fruits of WT, Lycopene-1, and Lycopene-5 (Figure 7). Unlike in WT, in Lycopene-1 and Lycopene-5, the plastid numbers in the pericarp cells were significantly higher at Br+7. Further, carotenoid-containing structures (osmiophilic globules) and crystal lines were higher in the plastids of lycopene mutant fruits than in WT fruits (Figure 7), resulting in the higher lycopene content of mutant tomatoes. In addition, the TEM images were different between the mutant and WT fruits at Br+7 (Figure 7). At the ripening stage, the vacuolisation of chloroplast was evident in the WT fruits, but a complete membrane was still observed. However, only plastids, but not chloroplast, were observed in the mutants. Our findings indicated that, with increased lycopene concentration in fruits, the conversion of chloroplasts to chromoplasts might occur earlier in transgenic fruits than in WT. This is sufficient to show that CRISPR/Cas9 multi-target genome editing can be successfully applied to regulating the metabolism of carotenoids to increase the accumulation of lycopene in the T0 generation of tomato. Lycopene content in tomato fruit subjected to genome editing was successfully increased to about 5.1-fold compared to wild type. Results suggested that CRISPR/Cas9 system can be used for significantly improving lycopene content in tomato fruit with advantages such as high efficiency and stable heredity.

Conclusion

Recent breeding approaches in solanaceous vegetable crops have leveraged advances in molecular biology, genomics, and biotechnology to enhance various quality traits. Molecular tools and genome sequencing techniques showed effectiveness to speed up the classical breeding methods for quality traits of complex pathways. Utilization of conventional breeding techniques along with powerful tools of biotechnology to pace the development program for redesigning of crops. Molecular markers, next generation sequencing, RNA interference (RNAi) and genetic engineering have great promise in reducing time and cost involved in breeding for improvement of quality traits in solanaceous vegetables. The integration of marker-assisted selection, genomic selection, genetic engineering, CRISPR-Cas9 technology, and participatory breeding holds great promise for developing varieties that meet the evolving needs of consumers, adapt to changing environmental conditions, and contribute to global food security. Continued collaboration between researchers, breeders and farmers is essential to overcome challenges and ensure the successful implementation of these innovative breeding strategies.

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