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Biochemical response of tomato accessions during different ripening stages

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Abstract
Tomato (*Solanum lycopersicum*) is one of the "protective foods" known for its special nutritive value and consumed all over the world as fresh and processed food. It is highly perishable commodity with limited shelf life. Storage duration and marketability highly depends on the fruit shelf life. To avoid post-harvest loss and to increase shelf life through genetic engineering, the current study focusses on the initial biochemical changes that occur during ripening and comparison of tomato accessions with green shoulder accessions (CBE SL.150). Tomato accessions CBE SL121, CBE SL130, CBE SL150 were studied for changes in lycopene, β-carotene, total phenolics and ascorbic acid contents through different ripening stages. Anti-oxidant assay (DPPH & ABTS) was also carried out at five ripening stages of tomato accessions. The result showed significant difference in biochemical changes among ripening stages as well as among the accessions. The result revealed that lycopene and β carotene increased through ripening stages i.e., immature to red ripe (1.416 to 3.569 mg/100g and 0.31 to 2.05 mg/100g). Total phenols increased with advancement of ripening stages and found to be cultivar dependent. Ascorbic acid was lowest at immature stage (18.30 mg/100 ml sample) while it expressed an increasing trend on ripening. Anti-oxidant assay showed similar results with low IC50 at red ripe which signifies greater antioxidant activity for both DPPH and ABTS. The results of this study provides us an insight on biochemical changes during different ripening stages and the influence of genetic makeup of tomato accessions.

Keywords: Tomato, ripening stages, biochemical changes, anti-oxidant activity

Introduction
Tomato being the most important vegetable is an abundant source of vitamins (Provitamin A, B1, B2, B3, C), carotenoids (lycopene, β-carotene), phenolics (flavonoids, p-coumaric acid, caffeic acid) and minerals (calcium, potassium, phosphorus, Sulphur, magnesium, iron). (Beecher, 1998) [2]. In India, tomato was cultivated in 778 thousand hectares with a production of 19,397 thousand metric tonnes (NHB, 2019) [18]. Andhra Pradesh ranks first in tomato production with 3146.96 thousand metric tonnes (NHB, 2019) [18].

Tomato fruit shelf life is a very important agronomic trait influencing storage duration and marketability. Fruit ripening is a complex developmental process which includes biochemical changes like accumulation of pigments, sugars, organic acids, degradation of chlorophyll, starch, solubilization of cell wall structure; metabolic changes like increase in biosynthesis, respiration, etc. which plays a significant role in nutritive value, color, aroma and flavor of fruit. (Bouzayen *et al.* 2010) [4].

Post-harvest loss of tomato accounts for 30.3% (FAO, 2019) [8] and is mainly because of immaturity, over-ripening, damage due to mishandling of machines and decay of harvested product. Increase in demand for quality product necessitates reduction in post-harvest loss. Tomato fruit quality mainly depends upon pre-harvest and post-harvest factor. (Arah *et al.*, 2015) [1].

From the germplasm maintained at Department of Vegetable Science, 30 genotypes were screened for yield characters and from that three accessions having highest yield were identified with better combining ability. In order to understand the biochemical changes that occur during different ripening stages comparison was made with green shoulder type. The finding of the study will help in developing the variety/hybrid with high yield, quality and better shelf life.

Materials and Methods

Plant material and growth condition

Tomato fruits for the present experiments were obtained from the Orchard, Department of Vegetable Science, Horticulture College and Research Institute, TNAU, Coimbatore. The plants were grown in well- drained sandy loam soil in open field condition and cultural practices were followed as given in the crop production guide for tomato (TNAU Agritech Portal, 2015) [28]. The experimental materials were tomato accessions *viz.*, CBE SL.121, CBE SL.130 and CBE SL.150, collected from germplasm at different ripening stages namely immature, mature green, breaker, turning and ripening.

Fruit characteristics

Fruits from the accessions CBE SL. 121, CBE SL.130, CBE SL.150 (Green shoulder) posses 3, 4, 4 locules respectively and average single fruit weight was recorded as 45, 63 and 56 g respectively. In addition, during different ripening stages Total Soluble Solids (TSS) was measured using hand held refractometer.

Chemical and reagents

Analytical grade acetone, petroleum ether, anhydrous sodium sulfate, sodium sulfate, oxalic acid, Sulfuric acid, 2,4 Dinitrophenyl Hydrazine (DNPH), Thiourea, 2,2, Diphenyl-2-picryl hydrazyl (DPPH), 2,2' Azinobis ((3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt) (ABTS), Potassium persulphate ($K_2S_2O_8$), Methanol, Catechol, Folin-Ciocalteu reagent, Sodium carbonate, Ethanol and Ascorbic acid were purchased from Sigma-Aldrich.

Lycopene and β- Carotene

Lycopene and β- carotene are carotenoids contributing for red and red orange pigment of fruits with naturally occurring antioxidant property. Lycopene and β- carotene content was determined using UV- visible spectrophotometer following the procedure given by Sadasivam and Manickam (1996) [23]. Approximately 1g of tomato samples were extracted with acetone followed by phase separation using petroleum ether to which 20ml of 5% sodium sulfate was added. Most of the lycopene and β- carotene present in upper petroleum ether layer was separated and the lower aqueous layer was re-extracted till it becomes colorless. To the pooled petroleum ether layer, 10g of anhydrous sodium sulfate was added, incubated at room temperature for 20 minutes and lycopene and β- carotene were measured at 503nm and 453nm respectively. Lycopene and β- carotene was estimated using

$$\text{Lycopene (in 100g sample)} = \frac{3.12 \times \text{Absorbance} \times \text{Total volume} \times 100}{\text{Weight of sample (g)} \times 1000}$$

$$\beta\text{-carotene (in 100g sample)} = \frac{\text{Absorbance of sample} \times \text{Total volume} \times 100}{0.2592 \times \text{Weight of sample} \times 1000}$$

Total phenolics

Total phenols measured using UV-Visible spectrophotometer. The phenolic hydroxyl group in sample reduces phosphomolybdic and phosphotungstic acid in Folin-Ciocalteau reagent resulting in blue color under alkaline condition. 1g of Tomato fruits were extracted with 80% ethanol and after that centrifuged and the supernatant was used as sample (0.2ml). 0.5ml Folin- Ciocalteu reagent incubated for 10 minutes followed by the addition of 1ml of sodium carbonate to standard and sample, incubated for few minutes till the color develops and measured the absorbance at 660nm. Pyrocatechol was used as standard. Total phenolics was expressed as mg of pyrocatechol.

Ascorbic acid

Oxidized form of vitamin C mainly dehydroascorbic acid is estimated. Ascorbic acid was extracted from 2g of tomato samples using 4% oxalic acid solution and the extracts were made up to 10ml with oxalic acid and titrated against 2, 6-dichlorophenol indophenol dye resulted in the appearance of pink color end product. Ascorbic acid was used as standard. Ascorbic acid in samples are expressed as mg of ascorbic acid equivalent.

$$\text{Amount of ascorbic acid} = \frac{0.5mg \times V2\ ml \times 100ml}{V1 \times 5\ ml \times Weight\ of\ the\ sample} \times 100$$

Antioxidant assay

Extraction of antioxidant

25g tomato puree was extracted with 80% acetone using blender and filtered with Whatman No.1 paper. Acetone was evaporated and the concentrated extract redissolved in 80% acetone and used as sample source. (modified protocol of Dewanto *et al.*, 2002) [7]

DPPH assay

Different concentration (20, 40, 60, 80, 100 mg/ml) of tomato extract were prepared from sample source and to that 4ml of DPPH solution was added. The decrease in absorbance was measured after 30 minutes at 517nm using UV-Visible spectrophotometer. 25mg DPPH dissolved in 100ml methanol was used for analysis.

ABTS assay

Different concentration (20, 40, 60, 80, 100 mg/ml) of tomato extract were prepared from sample source and to that 4ml of diluted ABTS solution was added and absorbance measured after 1 minute at 734nm. 5ml of 7mM ABTS and 88µl of 140mM potassium persulfate was mixed and incubated for 16 hours in dark which was further diluted to get absorbance of 0.70.

The percentage inhibition was calculated and results were analysed with reference to ascorbic acid standard graph.

Statistical Analysis

The results were statistically analysed with SPSS software. Significant differences were assessed by Duncan's Multiple Range Test with $p< 0.05$.

Results and Discussion

Lycopene and β-Carotene

In order to understand the biochemical changes that occurs during different ripening stages and to study the genotypic difference this study was undertaken. Tomato is a commercialized vegetable. It is being harvested at different stages to cater the need of consumers, for long distance transport, fresh market and processing etc. Carotenoids responsible for the pigmentation is an isoprenoid molecule, which in turn divided into lycopene and β-carotene. During ripening, in the plastoglobulin of chromoplast occurs the accumulation of lycopene and β-carotene. It acts as a visual maturation index of fruit ripening.

Mevalonic acid and DOXP (1-deoxy-D-xylulose-5-phosphate) pathway produces Isopentylpyrophosphate (IPP), whereas DOXP pathway is responsible for the formation of carotenoids. IPP and isomerized form of IPP- DMAPP

(Dimethyl allyl diphosphate) condense to form Geranyl geranyl pyrophosphate (GGPP). Two molecule of GGPP condense to form colorless phytoene catalyzed by phytoene synthase which in turn desaturated and isomerized to form lycopene by the action of desaturase and isomerase enzyme. δ-carotene and γ-carotene formed from lycopene by the action of lycopene ε-cyclase and lycopene β-cyclase, which in turn catalyzed by lycopene β-cyclase to form β-carotene and α-carotene (Su *et al.*, 2015) [27].

In our study lycopene and β-carotene content was found to increase through ripening stages *viz.*, immature to red ripe. The average β-carotene in immature stage is 0.31 mg/100g and in red ripe stage is 2.05 mg/100g (Table 2.) and lycopene content is 1.42mg/100g during immature stage and 3.57 mg/100g in ripening stage (Table 1.). The lycopene content was affected by maturity stages and time of harvest (Seymour *et al.*, 2012) [25]. Among the tomato accessions CBE SL.130 expressed highest β-carotene content of 2.73 mg/100g and highest lycopene of 4.63 mg/100g followed by CBE SL.121 and the accession CBE SL.150 recorded the least during ripening stage. The present investigation showed an increasing trend of β-carotene and lycopene from green to ripening stage. Similar increasing pattern was reported by Kotikova *et al.* (2011) [15] and Lavelli *et al.* (2000) [16] in both cherry and general varieties for β-carotene and Kaur *et al.* (2006) [14] for lycopene. In tomato the gene phytoene synthase 1 plays a key role in fruit ripening stage whereas phytoene synthase 2 mainly present in immature green fruit has no role in carotenogenesis in ripening stage (Bramley, 2002) [5]. β-carotene and lycopene was found to be significantly higher in CBE SL.121 and CBE SL.130 than CBE SL.150 (green shoulder variety). For β-carotene the percentage increase over CBE SL.150 at immature stage was 39.98% and 24.97% in CBE SL.121, CBE SL130 respectively whereas during ripening it was recorded as 53.42% and 60.02% respectively. The percentage increase of lycopene over CBE SL.150 was found to be significant. It increased from 24.74% to 38.63% through the ripening stages in CBE SL.121 and from 24.82% to 50.14% in CBE SL.130. The accessions CBE SL.121and CBE SL130 have significantly increased lycopene and β-carotene content and hence it can be used as one of the parent in breeding program to develop varieties suitable for food processing industry.

During immature stage β-carotene is rich in chloroplast and functions as accessory pigment in scavenging free radicals generated during photosynthesis and masks the yellow orange pigment. During ripening chloroplast is converted to chromoplast resulting in increased carotenoid content (Bhandari and Lee, 2016) [3]. Among carotenoids lycopene occupies about 85-90% over β-carotene (10-15%), this is due to downregulation of lycopene β cyclase which in turn upregulated lycopene content. The normal range of β-carotene and lycopene in red tomatoes is 3-12.5 to 10-15 mg/kg fresh weight. However, variation is observed among genotypes for carotenoid content (Ilahy *et al.*, 2019) [13]. Lycopene responsible for red pigment is a potent antioxidant which scavenges singlet oxygen with two times more efficient than β-carotene and 10 times more efficient than α-tocopherol (Horvitz *et al.*, 2004) [12]. As the ripening progress the carotenoids hyper-accumulate in plastoglobulin resulting in colorization of tissues in response to variation in molecular, developmental, harvest, post-harvest, genetic and environmental factors. The widely used method to improve carotenoid level in fruit is to introduce introgression of spontaneous and induced mutation in tomato (Ilahy *et al.*, 2019) [13].

Carotenoids not only contributes to fruit pigment but also act as a precursor for volatile compounds responsible for sensory characteristics. Because of its positive effects researchers focuses on engineering plants for enhanced carotenoid accumulation (Su *et al.*, 2015) [27].

Table 1: Estimation of Lycopene (mg/100g) content in tomato at different ripening stages

S. No	Tomato accessions (T) / Ripening stages (R)	CBE SL.121	CBE SL.130	CBE SL.150	Mean	%increase over CBE SL.150	
						CBE SL.121	CBE SL.130
1.	Immature	1.543	1.543	1.160	1.416	24.74	24.81
2.	Mature green	1.830	2.030	1.350	1.737	26.20	33.45
3.	Breaker	2.223	2.413	1.830	2.156	17.62	24.17
4.	Turning	2.603	3.280	2.030	2.638	21.99	38.05
5.	Ripening	3.763	4.633	2.310	3.569	38.62	50.14
	Mean	2.393	2.780	1.736			
		T	**R**	**T*R**		**T**	**R**	**T*R**
	SE(D)	0.028	0.012	0.063		0.22	5.05	1.12
	CD (0.05)	0.057	0.024	0.129		0.46	10.32	2.29

Table 2: Estimation of β- carotene(mg/100g) in tomato at different ripening stages

S. No	Tomato accessions (T) / Ripening stages (R)	CBE SL.121	CBE SL. 130	CBE SL. 150	Mean	%increase over CBE SL.150	
						CBE SL.121	CBE SL.130
1.	Immature	0.39	0.31	0.23	0.31	39.98	24.96
2.	Mature green	0.70	1.33	0.39	0.81	44.44	70.58
3.	Breaker	1.25	1.48	0.70	1.14	43.70	52.62
4.	Turning	1.33	2.34	0.86	1.51	35.27	63.32
5.	Ripening	2.34	2.73	1.09	2.05	53.42	60.02
	MEAN	1.20	1.64	0.65			
		T	**R**	**T*R**		**T**	**R**	**T*R**
	SE(D)	0.011	0.004	0.025		0.17	3.84	0.85
	CD (0.05)	0.023	0.010	0.052		0.35	7.84	1.74

Total phenolics

During fruit ripening tomatoes accumulate metabolites like phenolics, flavonoids, carotenoids and alkaloids. Phenolics is an important secondary metabolite synthesized via Shikimate

or Malonate pathway contributing for pigment, antioxidant activity, organoleptic attributes of fruits, as attractants of pollinators and repellants of pest, etc. (PA Silva *et al.*, 2019) [20]. In our study, as the ripening progress the total phenolic content increases and achieves a maximum during ripening stage. The average total phenolic among accessions were found to be 53.02 mg/100g during ripening and it was 20.20 mg/100g in immature stage. The normal total phenolic content in tomatoes ranges from 166-770 mg GAE/kg of fresh fruits. The variation in total phenolics mainly depends upon genotype of the cultivar, ripening stage and physical factors like light and temperature, pre and post-harvest conditions (Vallverdu *et al*., 2011; Hdider *et al*., 2013; Perea Dominguez *et al.*, 2018) [29, 11, 22]. In addition, total phenolic content show tissue specific variation in tomato fruits (Ilahy *et al.*, 2019) [13]. The percentage increase over CBE SL.150 ranged from 11.84% in ripening stage to 31.40% in immature stage of CBE SL.121 and 7.39% in ripening stage to 37.41% in immature stage of CBE SL.130(Table 3.). among the cultivars CBE SL.130 showed increased total phenolic content. Tomato fruit is a rich source of phenolics that contains 114 phenolic molecules in ripe tomato. Among phenolics, the main compound in tomato is chlorogenic acid occupying 75% in mature green and 35% in red ripe fruits. Other phenolics include caffeic acid, ferulic acid, p-coumaric acid, hydrocinnamic acid (Slimestad and Veruheul, 2009) [26]. Venkadeswaran *et al.* (2020) [30] reported high estimation of heritability and genetic gain in 24 cherry tomato genotypes for parameters like total phenols, TSS and lycopene, thus total phenolic content can be increased using conventional breeding method of selecting the best accessions from germplasm and crossing to produce new varieties or hybrids, in addition detecting the QTL, genetic transformation and candidate gene approach can also be used. In our study the accession CBE SL.130 showed increased phenolic content which can be used as one of the parent in breeding program.

Table 3: Estimation of total phenolic (mg) content in tomato at different ripening stages

S. No.	Tomato accessions (T) / Ripening stages (R)	CBE SL.121	CBE SL.130	CBE SL.150	Mean	% increase over CBE SL.150	
						CBE SL.121	CBE SL.130
1.	Immature	21.79	23.87	14.94	20.20	31.39	37.41
2.	Mature green	28.27	36.61	17.56	27.48	37.84	52.01
3.	Breaker	37.14	42.44	30.24	36.61	18.59	28.67
4.	Turning	42.32	47.62	33.21	41.05	21.46	30.20
5.	Ripening	56.13	53.45	49.46	53.02	11.83	7.38
	Mean	37.13	40.80	29.08			
		T	**R**	**T*R**		**T**	**R**	**T*R**
	SE(D)	0.367	0.473	0.820		0.32	0.81	1.62
	CD (0.05)	0.749	0.968	1.676		0.66	1.65	3.30

Ascorbic acid content

Ascorbic acid plays an important role as enzyme cofactor during photosynthesis and oxidized form of ascorbic acid namely dehydroascorbic acid act as an antioxidant during abiotic and biotic stress. Oms-Oliu *et al.* (2011) [19] reported that, dehydro ascorbic acid and iso citric acid content increased during ripening It is mainly distributed in cytosol and subcellular organelles like nuclei, chloroplast, mitochondria, vacuoles. Apart from that in apoplast it is present along with ascorbate oxidase, which non-enzymatically hydrolyze the cell wall resulting in fruit softening. The average ascorbic content in tomato was reported to be 12.6 mg/100g fresh weight but it varies with genotype, pre and post-harvest conditions, ripening stage and growing conditions (Ilahy *et al*., 2019) [13].

The average ascorbic acid content in our study during immature stage is 18.3 mg/100g and 34.37 mg/100g during ripening stage (Table 4.). The accession CBE SL.130 and CBE SL.150 showed on par ascorbic acid content which was followed by CBE SL 121. The ascorbic acid content was found to increase through ripening stage this is because during immature stage fruit undergo development like elongation and cell division, whereas during ripening the sugar, galactose accumulates. Galactose acts as a precursor for ascorbic acid and also during ripening large amount of free radicals released and it is being scavenged by increased amount of ascorbic acid (Ilahy *et al*., 2019) [13]. In addition to antioxidant activity it also functions as an enzyme co factor in modulating photosynthesis and activating other antioxidants (Gallie, 2013) [10]. Conventional breeding approach, genetic engineering, TILLING and New Plant Breeding Approach can be used to produce tomato fruits improved with ascorbic acid content.

Table 4: Estimation of Ascorbic acid (mg/100 ml) in tomato at different ripening stages

S. No	Tomato accessions (T) / Ripening stages (R)	CBE SL.121	CBE SL.130	CBE SL. 150	Mean	%increase over CBE SL.150	
						CBE SL.121	CBE SL.130
1.	Immature	16.74	18.75	19.42	18.30	-16.00	-3.63
2.	Mature green	17.41	21.43	20.76	19.87	-19.29	3.12
3.	Breaker	28.13	27.46	28.13	27.91	-0.07	-2.48
4.	Turning	28.79	32.81	31.46	31.02	-9.29	4.11
5.	Ripening	32.14	36.16	34.82	34.37	-8.34	3.72
	Mean	24.64	27.32	26.92			
		T	**R**	**T*R**		**T**	**R**	**T*R**
	SE(D)	0.232	0.100	0.519		0.31	3.64	1.56
	CD (0.05)	0.474	0.204	1.061		0.64	7.43	3.18

Total soluble solids

Total soluble solids (TSS) is a pre-requisite character preferred by consumers and food processing industries. Because high TSS contributes to sweetness, flavor and

organoleptic attributes of fruit and during processing due to high TSS it requires less addition of sugar and energy for processing (Sanchez *et al.,* 2020) [24]. As the ripening progresses starch gets converted to sugar as a result total soluble solids increases. The present investigation showed an average soluble solid content in immature fruit of 3.6° Brix and highest found in ripe fruit of 4.8° Brix. Among the accessions CBE SL.150 recorded highest TSS of 5.9° Brix followed by CBE SL.130 (5° Brix) and CBE SL.121 (3.6° Brix) during ripening stage (Table 5.). Similar variation in TSS was reported by Pal *et al.* (2018) [21] in 22 advanced tomato accessions with a range of 3.5 to 6.03° Brix. In breeding program single trait based selection leads to undesirable qualities in other trait. Hence Mulamba and Mock rank summation index of selection can be used as an efficient method for simultaneous selection of more than one traits (Sanchez *et al.,* 2020) [24].

Table 5: Estimation of Total Soluble Solids (° Brix) in tomato at different ripening stages

S. No	Tomato accessions (T) / Ripening stages (R)	CBE SL.121	CBE SL.130	CBE SL.150	Mean
1.	Immature	2.89	3.88	3.92	3.56
2.	Mature green	3.02	3.91	4.04	3.66
3.	Breaker	3.15	4.16	4.38	3.90
4.	Turning	3.42	4.67	4.79	4.29
5.	Ripening	3.60	5.00	5.90	4.83
	Mean	3.22	4.32	4.61	
		T	**R**	**T*R**	
	SE(D)	0.038	0.049	0.085	
	CD (0.05)	0.078	0.100	0.174	

Antioxidant assay

Tomatoes are rich source of antioxidants like ascorbic acid, vitamins, flavonoids and phenolics. Antioxidants mainly interact with free radicals, scavenges reactive oxygen species and play a key role in preventing chronic disease (Frusciante *et al*., 2007; Davies *et al.,* 1981) [9, 6]. ABTS and DPPH are free radicals used to determine antioxidant activity. In ABTS assay it determines antioxidant activity of fruit that scavenges ABTS free radical generated due to the interaction of ABTS with potassium persulfate. DPPH is also a free radical which undergo reduction upon interaction with antioxidant.

The antioxidant activity determined by ABTS assay recorded a highest IC50 in CBE SL.150 immature stage (48.35) and lowest IC 50 in CBE SL.121 (16.24) at ripening stage (Table 6.). IC50 showed decreasing trend through ripening process. The radical scavenging activity was inversely proportional to IC50. Ascorbic acid standard was found to be 12.35. as ripening progress flavonoids, phenolics, carotenoids increases as a result antioxidant activity is higher during ripening.

The ascorbic acid standard was recorded to be 54.23 in DPPH assay. In CBE SL.121 highest IC50 was recorded to be 148.14 and lowest in CBE SL. 150 (13.12). The radical scavenging activity was found to be inversely proportional to IC 50. The antioxidant activity mainly differs with cultivar and antioxidant assay method. Based on different extraction method antioxidant activity differs. Hence multifactorial approach is required to study antioxidant activity (Martinez Valverde *et al*., 2002) [17].

Table 6: Estimation of antioxidant activity in tomato at different ripening stages

S. No.	Antioxidant assay	ABTS (IC 50)			DPPH (IC 50)		
	Tomato accessions (T) / Ripening stages (R)	CBE SL. 121	CBE SL. 130	CBE SL. 150	CBE SL. 121	CBE SL. 130	CBE SL. 150
1.	Immature	35.08	41.77	48.35	148.14	91.13	88.45
2.	Mature green	30.69	40.73	43.03	94.88	65.48	69.11
3.	Breaker	26.70	33.97	34.42	88.59	28.71	24.73
4.	Turning	21.00	31.9	30.72	49.71	63.27	37.52
5.	Ripening	16.24	20.51	30.69	38.25	16.03	13.12
	Standard Ascorbic acid	12.35			54.23		

Conclusion

Tomato fruit quality and marketability depends on fruit color, chemical composition, texture and flavor which in turn contributed by lycopene, β-carotene, ascorbic acid, soluble solid content. Tomato fruit quality varies among cultivars, different ripening stages, cultivation and climatic conditions. In the present study the tomato accessions CBE SL130, CBE SL 121 and CBE SL150 (green shoulder type) are promising genetic resource for developing varieties/ hybrids. The accessions CBE SL150 is a green shoulder type which is a rich source of ascorbic acid content but comparatively with lesser β-carotene and lycopene. In conventional breeding, while selecting green shoulder accession as parent, the another parent should have high carotenoid content, which will improve nutritional quality besides the shelf life.

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