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Enhancing Health Benefits of Tomato by Increasing its Antioxidant Contents through Different Techniques: A Review

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Abstract

Tomato is known to be a great dietary source of antioxidant lycopene which is found to be linked with reduced risk of life-threatening diseases like heart attack and cancers. Antioxidants delay the aging process by mopping up reactive free radicals from cells, those if present may damage our DNA and other vital cellular organelles. Antioxidant metabolites are a group of vitamins, carotenoids, phenolic compounds, and phenolic acids that can provide effective protection against Reactive Oxygen Species (ROS) by neutralizing free radicals, which are unstable molecules linked to the development of many degenerative diseases and medical conditions. There are pre and postharvest techniques available in the literature and these when adopted by the researchers showed significant progress in enhancing antioxidant contents of tomato fruit. In addition, there are various biochemical and genetic modification approaches to improve the expression of several antioxidant enhancing phytonutrients, enzymes and genes in tomato fruit. Trichoderma enriched bio-fertilizer application in tomato enhanced ascorbic acid under the treatment of 100% bio-fertilizer and beta-carotene was increased under 75% Bio-Fertilizer+25% N whereas elevated lycopene contents were observed in case of recommended dose of NPK. Various omics approaches like genomics, transcriptomics, miRNAomics, proteomics, and metabolomics have emerged as extremely helpful tools for the plant scientists in improving the beta-carotene, lycopene and antioxidant levels resulting in highly desirable new tomato cultivars. Thus, in light of immense advantages of these techniques, the present study was undertaken to collect all the necessary information about different techniques employed by numerous researchers to increase the antioxidant contents in tomato and to document here the optimized experimental conditions that can be beneficial for future studies in this field. However, still in-depth genome wide studies are needed for better understanding and further enhancement of traits like flavor, quality and antioxidant contents in context to rapidly changing and uncertain climate.

Introduction

Tomato is a perennial plant and normally tomato fruit weighs 100-104 grams. "Tomato is one of the functional food crops with more than 181 million-tons production from 5 million hectares harvested area in the world (FAO, 2019)". In recent years tomato crop is valued for its nutritional and health benefits. Tomato species (Solanum lycopersicum L.) is one of the most consumed vegetables in the world due to its rich supply of essential nutrients especially antioxidants. Regular intake of tomato weather fresh or in processed form in the required amount can decrease the danger of multiple diseases [1,2]. Tomato paste can also be a good source for important antioxidants such as flavonoid and phenolic compounds for human body [3]. Thus its consumption has major contribution in increasing level of antioxidants and fibers in the body and even waste of tomato can be a good source of antimicrobial activities [4]. The method of growing different cultivars of tomato, their storage conditions and time of harvesting can affect the level of antioxidants [5-8]. Cherry tomatoes are increasingly demanded now a days [9], and despite their small size they can be rich in antioxidants [10,11]. Same tomatoes harvested at a different time period may contain variable level of vitamin-C [12]. The antioxidants induce a protective response against oxidative activity in the fruit as well as the human body which may occur in response to various environmental factors [13]. Tomatoes contain different types of antioxidants carotenoids, flavonoids, Vitamins especially vitamin-C and lycopene, polyphenols and anticancer substances while carotenoids are most abundant and medically valuable [14-16]. Carotenoids have a major role in human health and nutrition due to their anticancer properties [17]. Carotenoids provide red color to the tomato and other fruits. Mostly two types of carotenoids are reported in tomatoes i.e. lycopene and β-carotene, lycopene has major contribution >80% carotenoid content in tomato fruit and is responsible for the red color of fruit while β-carotene contents are 7-10% and is responsible for orange color in the product [18]. Considering the enormous health benefits of tomato fruit and its medicinal value of its constituent chemicals, this effort has been undertaken to review the latest published research in order to determine the antioxidant properties of tomatoes and its beneficial effects for human health and vitality.

Methods

Literature search strategy and selection criteria

Literature included in the present review was searched and collected from most authentic, well reputed and high impact factor international and national journals and databases like AGRICOLA, AGRIS, SCOPUS, Web of Science, PubMed, ResearchGate etc. Recently published papers that were not older than 10 years were selected for review and citation. Various powerful search engines like Google Scholar, Microsoft Academics, Worldwide science etc. were employed for searching the most relevant research material from web.

Discussion

Importance of antioxidants for humans

Antioxidants are high value biochemical compounds found in tomatoes in very high concentrations and play an unequalled role in fight against oxidation process which may result in cellular damage or death [19]. Lycopene is an effective antioxidant and used as fortified nutritional supplement [20]. Its amount in the tomato fruit can be increased by applying pulse light treatment to tomato, with wavelengths of emitted spectrum ranging from UVC to infrared (180-1100 nm) and for several minutes [21], and also by adding yeast and bacterial genes in case of transgenic varieties. Antioxidant compounds play an important role to prevent the oxidation of oxidizable products [22], and also have an impact on the protective system of the body in response to Reactive Oxygen Species (ROS), that are hazardous by-products produced during normal aerobic cellular respiration [23]. Tomato sauce can be a good source for the intake of antioxidants [24]. Results from clinical studies showed that regular intake of lycopene can reduce the risk of various types of cancers of vital human organs like prostate, lung and stomach [25] as well as reduces the risk of a life threatening medical conditions like coronary heart and kidney diseases [26]. Antioxidants also play a vital role in prevention of Ischemic brain stroke disease [27]. Antioxidants have become very sort after component of cosmetics industries especially natural antioxidants taken from seaweed are very demanding and popular in the beauty industry [28]. Antioxidants can donate electrons to stabilize ROS and to prevent their detrimental effects, including both endogenous (are synthesized within body and by the body itself) and exogenous molecules (those which are from external sources to the body)[29]. Consumption of fruit-based antioxidants are found to be helpful for patients suffering from bowel disease [30]. Research has confirmed that many diseases like diabetes, different kind of cancers, Parkinson's disease, cardiovascular diseases [31] and Alzheimer's diseases are highly interrelated with cellular redox and free disproportion [32] and to maintain homeostatic balance in the human body as well as to achieve the prevention and cure of diseases, the consumption of antioxidants in our daily life has become necessary [33]. Most importantly flavonoids play vital role in curing the disease like Atherosclerosis by providing anti-inflammatory properties and due to this reason flavonoids should be an integral part of the human daily diet. Such defensive factors might also include fibres, trace minerals, and many other micronutrients which include many of the vitamins, pro-vitamins and various other compounds that have chemical properties of antioxidants [34]. It is hypothesized that various factors in the changing diet of migrants are related to increased risk of cancer that may include food-based carcinogens and various other cancer-promoting elements like high levels of body fats [35]. Many antioxidants have been described to exert numerous advantageous effects on human health that include antiviral, anti-cancer, cardio-protective, antibacterial, anti-inflammatory and neuro-protective properties [36-38]. Including fruits and vegetables in your diet can increase volume of antioxidants in the blood stream up to significant level [39]. It has been proposed that a reduced amount of antioxidant activity in the cell can result into elevated risk of cancer that is why the ingestion of antioxidants can be helpful to prevent carcinogenesis [40]. Tomato is a nutrient-condensed food that offers a wide range of benefits to various bodily systems. Nutrients present in the tomato are beneficial for healthy skin, weight loss and for cardiac health. Tomatoes having high antioxidant contents should be included in the diet because it can help to protect human body against cancers, maintain normal blood pressure and decrease the levels of blood glucose in diabetic patents [41]. It is estimated that 35% of the deaths occurring in the USA due to cancer are related to poor diet. Therefore, dietary modification is a practical strategy to prevent the chronic diseases in human. The real aim of Recommended Dietary Allowances (RDA) has been shifted from prevention of clinical deficiencies to be focused on the prevention of diseases such as birth defects, coronary heart disease and cancer [40]. Although the concentration of carotenoids is less in tomato as compared to the other vegetables, none the less, tomato stands among the top vegetables as a source of vitamin A, E and C due to its high consumption all over the world. Although tomato is a rich source of many nutrients, secondary metabolites, organic acids and vitamins etc. but it is important that after picking tomato should be consumed as early as possible because after detachment from the plant they continue to ripen and reach a point where the quality deteriorate to an extinct to render them useless for consumption. Interestingly according to latest findings the antioxidants are also known to be very effective in the treatment of hypertensive patients of Grade I & II with no reported side effects [42]. One tomato fruit can provide approximately 20% of vitamin A and 40% of the recommended daily intake of vitamin-C [43]. The removal of the fruit skin is very detrimental, as it has been found that by removing the tomato peal can result in the loss of 80% lycopene, 63% of phenolic compounds and 57% of β-carotene[44].

Importance of antioxidants in plants

Antioxidants greatly delay or reduce the oxidative stressors in plants [45]. These antioxidants are produced in vivo, i.e., superoxide dismutase (SOD) and decreased glutathione (GSH) etc. or utilized as diet based antioxidants [45]. Various plants have been known as a source of dietary antioxidants. It is estimated that almost two-third of global plant species are of some medicinal value and almost all these plants produce antioxidants [46]. The importance of the exogenous plant antioxidants was first highlighted with the finding and extraction of ascorbic acid in plants. Presently there are about 19 in-vitro and 10 in-vivo approaches of estimating antioxidant contents in plants [47]. Mostly in-vitro methods have been reported to show strong antioxidant activity in plants. This may be due to their unique ability to produce non-enzyme antioxidant substances like AsA and glutathione, in addition to secondary metabolites like phenolic complexes as shown in figure-1. Researcher have found that in a plant cell, chloroplast and mitochondria are the key power-generators, as well as sites of ROS production. Similarly, peroxisomes are known to be 3rd most prominent site for the synthesis of ROS, i.e., hydrogen peroxide (H2O2), superoxides (O2-) and nitricoxides (NO). Likewise ROS, other compounds like reactive nitrogen species (RNS) like nitric oxides (NO) are also produced in different cell organelles that include chloroplast, mitochondria and peroxisome [48]. These free-radicals are constantly synthesized in plant cells due to genetics or in response to stress as signaling molecule [49,50]. The over synthesis of these free-radicals in plants can lead to damaged DNA, cellular protein and lipids. The antioxidant response is considered to be a very important process in the protection of plants against oxidative damage which is caused by a great number of environmental factors such as light, climate, salinity, temperature, and nutritional deprivation etc. [51]. Among these factors, the oxidative stress is apparently the main cause of membrane damage and changing the composition and contents of antioxidant compounds that results in the overall change in antioxidant activity of the tissue [52]. Increased activity of antioxidant pathway is one of the main mechanisms found to be involved in the resistance against stresses like chilling, heat, drought, salinity and wounds. Antioxidant defense system in tomato can be strengthen by delaying biosynthesis processes through the application of selenium [53] and bio-augmented compost [54]. During the days of peak durations of solar radiation and in extreme cold weather, plants are forced to develop a defensive mechanism against the ultraviolet (UV) radiation and extreme production of free radicals by internal accumulation of antioxidant substances [55]. A detailed stress perception and antioxidant defense mechanism in plants is shown in Figure-1. Tomato is one of the most commonly consumed fresh as well as processed vegetables around the globe for its nutritional value and bioactive antioxidants such as phytosterols [56], carotenoids, phenolics and vitamins C and E [11]. It is reported that some cultivars of tomato have more percentage of phenolic compounds than other widely grown varieties. The Portuguese ex-situ conserved germplasm of tomato is prominent for its high antioxidant concentrations [57].

Cultural and molecular approaches to enhance antioxidants in tomato

Followings are the pre-harvest and post-harvest techniques through which antioxidant contents can be increased in tomatoes.

Aeroponics:

This is the process of growing plants in a medium without soil, but roots are kept suspended and sprayed with water or nutrient solution [58]. Fruit bearing tomato plants were grown in a controlled environment under LED with light intensity of 250 µmolm-2s-1 at the top of plant canopy. Five plants were planted per meter square (m^{-2}). Temperature was kept between 25°C±3 during the light period and during the dark period, temperature was maintained at 18°C±3. The CO2 level was the same as the outside atmosphere. The modified Hoagland nutrient solution was the basic culture medium. The architecture of aeroponic system was designed so that it can be easily fitted within the top of the growth trays. The system included two misters spaced at 20cm intervals. Misters were angled downwards to ensure coverage of the plants roots throughout the entire growth cycle. Excessive aeroponic water was collected at the bottom of the slightly angled channel and flows out back to the petal reservoir. Tomato seeds were germinated at 24°C for days and then tomato seedlings were transplanted to the aeroponic system. The activity of the two important antioxidants i.e., peroxidase and catalase were measured in tomato leaves at ontogenesis. It was observed that during early leaf development, POD and CAT activity increased and reached its maximum values at the development stage while decreased later at leaf senescence. Also, those plants that were grown in porous tube vermiculite condition throughout the leaf development showed low levels of enzymatic antioxidant activity. Similarly, higher lycopene contents were noted in aeroponics grown tomatoes as compared to vermiculate based apparatus. No significant change in beta-carotene contents was observed among both these treatments [58]. A graphical representation of an aeroponics system is shown in Figure-2.

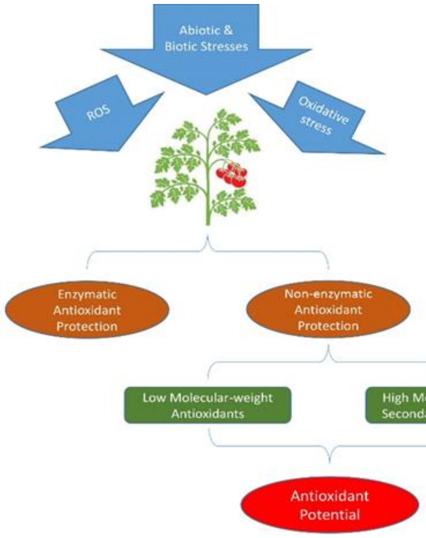

Figure 1: Diagrammatic representation of stress perception and antioxidant defense mechanism in plants.

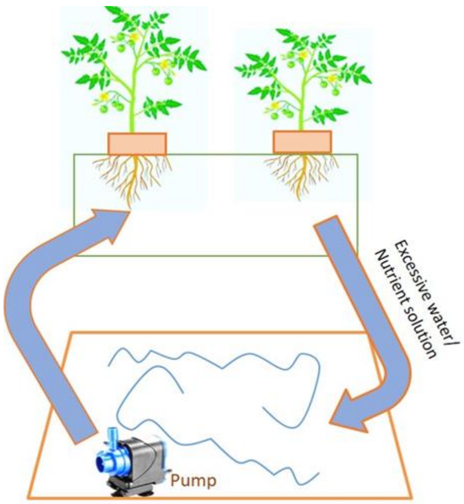

Figure 2: Detailed representation of a model aeroponic system.

Application of salicylic acid

Healthy, uniform and matured green tomatoes were harvested, washed, and dried. Salicylic acid treatment with 4+1, 4+2, 4+4 mM concentration were applied through plant foliar method three weeks prior to the harvest. After harvest fruits were dipped for five minutes in salicylic acid solution for five minutes. Treated and non-treated tomato fruits were stored at 10° C with relative humidity between 85-90% for 10-40 days respectively. Vitamin C or ascorbic acid contents were measured using titrimetric method and result was expressed in mg/100g [59].). After 40 days of storage it was observed that the treated tomatoes with pre and post-harvest treatment of salicylic acid contain more ascorbic acid contents as compared to the normal tomatoes and results were significant ($p<0.001$) [60]. A diagrammatic representation of this approach is shown in figure 3.

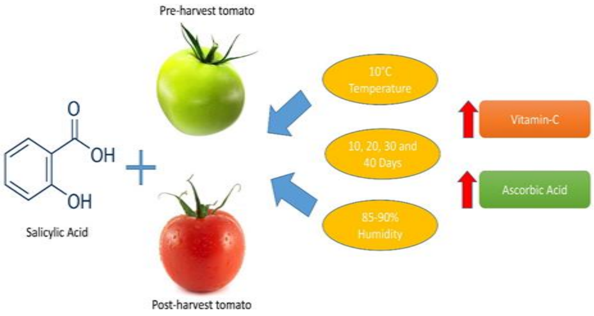

Figure 3: Foliar application of salicylic acid on pre-and post-harvest tomato fruit.

Application of Trichoderma enriched bio-fertilizer in soil

Antioxidants present in tomato are very useful in preventing cardiovascular diseases. Application of bio-fertilizer has significantly increased lycopene and vitamin c contents of tomato. Seeds were collected to grow, and land was prepared having the seedbed size of 2.5×5.0m. First the nursery was grown and for the protection of seeds from rain and excessive fog the polythene bags were used after the 25 days. Healthy seedlings were transplanted with a spacing of 50×50 cm [61].). It was ensured that Trichoderma enriched biofertilizer was prepared by well reputed agriculture enterprise as bio-fertilizer having good quality cow dung, poultry litter, household/kitchen waste and press mud of sugar mills in its constituents. In this technique, 6 treatments were used. T1 was controlled (without NPK and Bio-F), T2 was recommended dose of NPK, T3 was 100% Bio-Fertilizer, T4 was 75% Bio-Fertilizer +25%N, T5 was 50% Bio-Fertilizer +50%N, T6 was 25% Bio-Fertilizer+75%N and recommendable dose of phosphorus and potassium was used in last 3 treatments (Figure-4). And NPK contained TSP (triple superphosphate), urea and MOP (muriate of potash) and a full dose of all these comprised the bio-fertilizer at the final preparation of land. Nitrogen was applied in 3 splits. Ripened tomatoes were harvested, weigh and expressed in kg per plant. Beta-carotene and lycopene were measured at spectrophotometer (Hitachi no. 200-20, Hitachi, Japan) by using method described by [62]. According to observations, the ascorbic acid was increased under the treatment of 100% bio-fertilizer (T3) and beta-carotene was increased under T4 (75% Bio-Fertilizer+25% N) whereas enhanced lycopene contents were observed in case of T2 (recommended dose of NPK). These results proved that quantity and function of antioxidant in tomato were greatly enhanced due to the application of bio-fertilizer [40].

Freeze drying

In this procedure the tomatoes were first cut into slices having size of 1×1cm2 and spread on the steel tray and then dried in a freeze drier at the vacuum pressure of 0.1333 mbar for 24 hours at -50° Celsius [63]. The extract of aqueous sample for the determination of antioxidant capacity was prepared according to the already optimized protocol [21] where 3ml of 75% methanol was added to 0.5-1g sample of tomato slices and sonicated for 15 min and later sample was centrifuged for 10 min at 2500rpm to collect the supernatant. Then 75% (3ml) of methanol was added to pellet and procedure was repeated to make the final volume of 10 ml and both supernatants were combined. It was observed that GSH, cysteine, total phenolic compound and CUPRAC values were increased by this freezing drier method [64]. Hence by keeping tomatoes in cool environment can significantly increase antioxidants levels in fruit [65].

Exposure of tomatoes to infrared light at green stage

Freshly picked first fruits from the first truss were placed on plastic tray (42 fruits per each tray) and covered with aluminum foil to ensured that fruits were not in contact with each other. Trays were stored in custom built climate chamber for 20 days in the first experiment and for 14 days in second experiment under the constant day/night temperature (20°C/19°C) and relative humidity (RH 75%-85%). In experiment # 1, the effect of duration of red light radiation on the accumulation of health promoting compounds was noted [66]. Whereas in experiment # 2 the effect of continuous or intermittent red light was measured on the accumulation of health promoting compound. In both of these experiments tomatoes of the control treatment were kept in dark (having same RH and temperature). For red light treatment tomatoes were irradiated with light emitting diode (LED) module which was installed in the climate chamber [67] [68]. The experimental settings were controlled by equipment specific software. Tomatoes undergone this process required five less days to reach the same maturity levels. Moreover, this exposure raised concentrations of lycopene, total flavonoids, phenolics and β-carotene in the cut fruit sections. The light treatments method proved to be simple and environmentally safe technique to improve the health-promoting antioxidants.

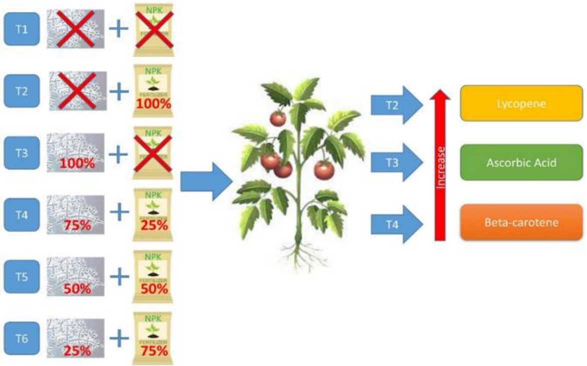

Figure 4: Soil based application of Trichoderma Enriched Bio-fertilizer.

Metabolite engineering

The metabolic engineering in plants involved various biochemical and genetic modification techniques to enhance the expression of several phytonutrients, enzymes and genes in tomato fruit. Ascorbic acid (Vitamin-C) is a vital dietary phytonutrient required to perform major metabolic processes in human body [69]. To enhance the ascorbic acid (AsA) production in tomato, scientists overexpressed the genes associated with ascorbate recycling enzymes DHAR and MDHAR. The green and ripened fruit of resulting transgenic lines showed 1.6-fold increase in AsA levels thus enhancing its nutritional value. β-carotene and lycopene are well-known for being beneficial for human health. When a carotenoid (crtl) gene of bacterial origin was transformed in tomato, an increase of 3-fold (45%) of β-carotene level was observed in transgenic lines [70]. Folate is a vital nutrient and its deficiency in humans can cause many neurological and physical defects and diseases. In biological system the folate is synthesized from p-amino-benzoate (PABA). Plant scientists managed to increase the level of folate in tomato fruit by 25 folds by overexpressing the amino-deoxychorismate-synthase which is 1st enzyme of PABA pathway [71]. Beta-carotene is also a precursor for Vitamin-A, and a powerful antioxidant. When a lycopene B-cyclase gene from flowering plant daffodil was transformed in chloroplast of tomato, more than 50% increased β-carotene contents was observed in transgenic tomato plants [72]. Other studies involving antioxidant enhancements showed that when a Vitis vinifera L. stilbene synthase (StSy) gene was overexpressed in tomato plant, the transgenic plants relatively accumulated more transresveratrol, resulting in much higher contents of glutathione and ascorbate which are vital soluble antioxidants involved in crucial metabolic activities in humans [73]. In the effort to improve the dietary value of tomato fruit scientist have utilized unique techniques like RNA interference (RNAi). Attempts were made to suppress an endogenous photo-morphogenesis gene (DET-1) in tomato fruit with the help of RNAi technology. Resulting transgenic plant indeed contained degraded DET-1 expression while consequently an impressive increase in both flavonoid and β-carotenoid contents was observed thus improving the nutritional value of genetically modified tomato [74].

In addition to the advanced metabolic and genome editing and engineering techniques, many researchers have also adopted conventional breeding techniques to improve antioxidant contents of tomato fruit. The detail of the approaches adopted, specific technologies used, and genes/pathways improved are described in Table-1.

Omics approaches

With the introduction of various omics approaches in plant sciences, exceptional advancements have been made in study of signaling pathways involved in beneficial metabolites synthesis across diverse species. Omics approaches such as genomics, transcriptomics, miRNAomics, proteomics, and metabolomics have changed the landscape of different diseases including stroke, diabetes, and cancer. Genomic approaches such as genome wide association studies have led to the identification of 30 loci, which were used in establishing relationship among body mass index and the risk of obesity [1].

Transplastomic tomato plants possessing LCYB gene from daffodil plant were engineered using biolistic gene gun. The results showed that introduction of a single gene of β-carotenoid biosynthetic pathway in diverse tomato genotypes induced considerable metabolic modifications in carotenoid, apo-carotenoid and phyto-hormonal pathways resulting in up to 77 percent yield increase and improvement in fruit's vitamin-A contents [2]. During fruit maturing, lycopene accumulation inhabits the production of cyclical carotenoids. Fruit-targeted over-expression of lycopene-beta-cyclase (LCYb) lead to enhanced beta-carotene (pro-vitamin-A) contents in tomato fruit [3]. Carotenoid biosynthetic pathway regulation and limitations in its accumulation in the fruit are not yet fully understood. Apel and Bock, 2009 [4] over-expressed the lycopene-beta-cyclase genes from Narcissus pseudonarcissus in the tomato chloroplast. It was also observed that expression of plant-based gene was able to synthesis lycopene better that the one having bacterial origin. the conversion rate of lycopene into beta-carotene was noted at 1mg/g of dry weight that is almost 50% increase in total beta-carotenoid in the transplastomic tomato.

Micro-RNAs are small (19 to 24 base pair long) non-coding RNA units that can strongly alter gene expression. By binding with their target mRNA these short RNA fragments can cause cleavage at specific translational sites hence, disrupting the gene expression. Lab based studies have shown that tomato-based lycopene are alimentary anti-cancerous agents. Lycopene is known to alter testosterone metabolism in the body. Testosterone plays key role in prostate cancer development through the activation of androgen bio-signaling pathway. The study showed that the miRNA expression was down-regulated in transgenic TRAMP mice in comparison to wild-type mice in initial prostate cancer stage while lycopene played minimum roles in this process [5]. Xu et al. (2010) [6] also reported the regulation of fruit-based carotenoids by miRNAs. He revealed 51 known and 9 new differentially expressing miRNAs from a sweet orange mutant through genome sequencing. Among these TC5 gene was found to encode lycopene beta-cyclase (LYC-b), a rate-limiting enzyme in the conversion of lycopene to downstream cyclic carotenes. Koul et al. (2016) [7] during a comparative study found key regulatory genes linked with carotenoid biosynthesis that include mi-R1911, mi-R482, mi-R172, mi-R396, mi-R395, osa-mi-R169i-5p.2, and ppt-mi-R1027a.

During fruit maturity, beta-carotenoid accumulation not only decide the level of antioxidant levels but also its colors and shelf life. For decades the plant breeders are deploying concerted efforts in order to improve on these particular traits in tomato fruit. the evidence from past studies have shown a strong relation between CYCB gene expression and color development in fruit. Similar work has identified a single mutation in SGR sequence that hinders chlorophyll depletion thus resulting in retention of brown color in a few tomato varieties. By crossing orange KNY2 and brown KNB1 tomato genotypes, the scientists were able to produce a novel orange brown F2 generation. This orange-brown fruit possessed higher beta-carotene and chlorophyll contents than the parental lines. Such studies have proven to be very helpful for the plant breeders in order to develop tomato varieties have desirable fruit color with added benefits of enhanced beta-carotene and antioxidants [8].

Some less utilized techniques for antioxidant improvement

Following are some additional but less known methods of improving health promoting antioxidants that were developed and tested for enhancing the health benefits of tomato:

i. Steam cooking [103].
ii. Addition of herbs while cooking [104].

Bioavailability of antioxidants for absorption

Tomato and its by-products are the richest source of lycopene for humans as its fruit contains a significant number of beneficial antioxidants such as phenolic flavonoid and ascorbic acid etc. However, it is observed that in fresh tomatoes the anti-oxidants components greatly vary with type of cultivars, growth conditions, harvesting time and temperature. By measuring the concentration of plasma and urine after ingestion of tomatoes as whole or in a processed form, the bioavailability of some carotenoids including phenolic compounds have been described here.

Fruit sampling and in-vitro digestion

Tomatoes fruit from three commercial tomatoes cultivar were used in study which is mostly used for the fresh consumption. Tomatoes were grown hydroponically on a commercial green house and harvested at red ripe stage. The modified method of miller was used to study the in vitro digestion of tomatoes. The method consisted of a pepsin-HCl digestion for 2 hours, followed by a pancreatin digestion with bile salt for further 2 hours at 37°C. After the completion of in vitro digestion, total phenolic, flavonoid, lycopene, ascorbic acid and antioxidant activities in the fresh tomatoes as well as in the digestion extracts and residual tomatoes solids was analyzed. In-vitro digestion can cause a serious amount of decrease in antioxidant activity up to 75% even in fried form [105].

Measurement of total phenolics

Total phenolics was measured by using the Folin-Ciocalteau method. Hydrophilic, Lipophilic and Digestion extracts were properly diluted with 2.5ml freshly diluted 0.2M Folin-Ciocalteau reagent. By adding 2ml of 75% w/v Na_2CO_3 reaction was neutralized, samples were vortexed for 20 sec then incubated at 45°C for the 15 min and resulting absorbance of blue color was measured at 765nm on UV-visible recording spectrometer. Gallic acid was used as standard and result was expressed as Gallic acid equivalents. The total phenolic results were corrected for the contribution of ascorbic acid [56].

Measurement of lycopene

Lycopene from homogenized tomatoes samples digestion extracts and residues tomatoes solid was extracted in a mixture of hexane, octane, and ethanol. The absorbance of the hexane solution containing lycopene was measured at 400nm on a spectrometer and specific extinction coefficient was used to calculate the concentration of lycopene [56].

Approach	Technique	Gene	Pathway	Reference
Metabolite engineering	Modifying key-regulatory pathways	*GDP-mannose-3,5-epimerase.* *Mdhar enzyme* *Dhar enzyme*	Ascorbic acid (AsA)	[69,75]
		Bacterial- phytoene-desaturase gene (crt-I)	β-Carotene	[70]
	Novel gene expression	Yeast-GMPase gene	Ascorbic acid	[76]
		Bacterial *GCHI* gene		
		ADCS gene from Arabidopsis p-amino-benzoate (PABA)	Folate	[71,77]
		Erwinia herbicola lycopene b-cyclase	Carotenoid	[72]
		Vitis vinifera StSy gene	Resveratrol	[73]
	Gene silencing technology	*Deetiolated-1 (DET-1 gene)*	Anthocyanins	[78]
		Del and *Ros-I*	anthocyanin biosynthesis	[79]
		SlMYB-12 gene	Flavonoid naringenin chalcone	[80]
Genome Editing	CRISPR/Cas9	Psy1 and CrtR-b2	carotenoid biosynthesis	[81]
		Knock-down of carotenoid metabolic pathway genes	lycopene biosynthesis and accumulation	[82]
		Suppression of SlMPK20	Mitogen-activated protein kinases (MAPKs)	[83]
		phytoene desaturase gene	γ-aminobutyric acid (GABA)	[84]
		anthocyanin biosynthesis	ectopic accumulation of pigments in tomato tissues	[85]
		Glutamate decarboxylase (GAD) genes *SlGAD2* and *SlGAD3*	γ-aminobutyric acid (GABA) biosynthesis	[74]
		Inhibition of 5 genes involved in lycopene to β- and α-carotene conversion	biosynthesis of lycopene by	[86]
Conventional breeding	Association mapping QTL	29 SSRs and 15 morphological characters	Anthocyanin	[87]
		Lyc-7.1 and *lyc-12.1*	Lycopene	[88]
	QTL linkage mapping	Differences during the plant growth stages	Numerous components	[89]
			AsA, phenol, and solvable solids	[90]
			Total phenols and Bx-level	[91]
	Quantitative trait locus (QTL) mapping study	High-performance liquid chromatography (HPLC), spectrophotometry, and colorimetric assays. At chromosome 7 & 12	Lycopene	[92]
	Genetic mapping of single-locus and epistatic quantitative trait loci (QTL)	*FrW7-1*	fruit size QTL co-localized with QTL involved in soluble solid, vitamin C	[93]
		1-deoxy-D-xylulose 5-phosphate synthase and Tocopherol cyclase	beta-carotene and vitamin C	
	Dynamic quantitative trait loci (QTL)	SSR146 for Chromosome no 4g	Lycopene	[94]
		SSR110 for chromosome no 9		
	Linkage mapping through genome wide SSRs	SSR and SNPs Linkage group 11 *(LG11)* QTL ID *11.1*	Lycopene	[95]
	QTL analysis using introgression lines	QTL *(bc6-2 and bc6-3)*	β-carotene	[96]

Table 1: Various Multi-Dimensional approaches adopted for the improvement of antioxidants in Tomato.

Measurement of antioxidant activity

The radical scavenging capacity (antioxidants activity) of solvent and digestion extracts was measured by using the modified 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS) radical decolorization essay. Manganese dioxide was generated with ABTS radical. Assay was performed with three dilutions per extract and duplicate reading was taken from each dilution. Trolox (6 Hydroxy-2,5,7,8- trimethyl chroman – 2 – carboxylic acid), a water-soluble vitamin E analysis was used to prepare the standard curve and activity was reported in the micromolar Trolox equivalent antioxidant capacity (micro mol TEA) [106].

Conclusion and Future Prospects

Undoubtedly tomatoes are a good source of antioxidants and play a significant role in delaying the ageing process in human beings. Lycopene is one of the most potent antioxidants in tomato with a great protective potential against human diseases of skin, bone, liver and brain as reflected by various studies. There are certain methods that showed significant achievements in improving the antioxidant contents of tomato fruit like aeroponics, salicylic acid application, supplementation of Trichoderma-enriched biofertilizer, microwave heating, and freeze drying, etc. Researcher have also employed advanced techniques like biochemical and genetic modification to enhance the expression of several phytonutrients, enzymes and genes in tomato like Vitamin-C, ascorbic acid (AsA), β-carotenes and lycopene etc. These techniques involve modification of key-regulatory pathways, gene silencing, novel gene expression and genome editing technologies that are not only stat of the art but also very precise and less time consuming. However, further investigations are required to reveal the underlying mechanisms especially mode of gene expression of lycopene related primary and secondary metabolic pathways. Additionally, in context of rapidly changing climate, improvement of tomato flavor, quality and antioxidant contents will be a tough challenge in the future. A genome wide association mapping will give an insight into the genetics of these traits and will make way for better understanding and improvements in genetic infrastructure of this vital fruit.

Author Contributions

Ammad abu Haraira: Conceptualization, Investigation, Formal analysis; Hafiz Sabah-ud-din Mazhar: Conceptualization, Writing-review & editing; Abdul-Rehman Tahir: Writing-review & editing; Afrasyab Ahmad: Review & writing; Imran Habib: Writing-original draft, Visualization, graphics & editing; Shahid Nazir: Review & writing and proof reading; Muhammad Tariq: Proof reading.

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