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Bioactive compounds of tomato fruits from transgenic plants tolerant to drought

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ABSTRACT

Advances in agricultural biotechnology bring forth the need for experimental evidence for benefits and risks of engineered crops and the quality of fruit products obtained from them. Tomato fruits from non-transgenic (NT) and *BcZAT12*-transformed tomato lines ZT1-ZT6 (cv. H-86, var. Kashi vishesh) tolerant to drought, were assessed for nutritional quality, changes in physico-chemical characteristics and health-related bioactive compounds. Fruits from transgenics were evaluated for size, pH, total-soluble solids, total sugars, phenolics, flavonoids, vitamin C, lycopene and β-carotene. An early ripening of tomatoes from transgenics with more red but smaller fruits, high sugar levels, elevated phenolics, flavonoids, lycopene and β-carotene with an unaltered vitamin C levels as compared to tomatoes from non-transgenics were noted. Results suggest that although differences between tomatoes from transgenics and non-transgenics do exist, yet tomato fruits from transgenic plants have relatively improved antioxidant capacity than those from non-transgenics and therefore may be products safe for consumption.

1. Introduction

Plant genetic engineering provides powerful tools to enhance plant modification for potential benefit of society (Falk et al., 2002). Modifications that protect the crop from biotic or abiotic stress benefit the producer. The development of crops containing single gene transfer are controllable, testable and predictable therefore, the majority of modified crops lie in this group (Falk et al., 2002). The conferring of plants with genes that help them withstand a wider range of environmental conditions helps increase productivity. Tomato fruit forms an important part of daily diet, thereby contributing to its demand in local and worldwide markets. Tomato (*Solanum lycopersicum*) consumption has recently been demonstrated to be beneficial to human health, because of its content of bioactive compounds such as carotenoids, β-carotene (precursor of vitamin A), ascorbic acid (vitamin C), phenolic compounds namely flavonoids and phenolic acids, tocopherols (vitamin E) and many essential nutrients (Soto-Zamora, Yahia, Brecht, & Gardea, 2005). The nutritional value, color, flavor of the fruits and their products depend mainly on content of lycopene, β-carotene, ascorbic acid, sugars and their ratio.

Baldwin et al. (1998) showed that sugars were positively correlated with overall flavor acceptability of tomato by consumers. Soluble sugar is an osmolyte in plants and accumulates in response to drought stress. Generally two type of sugars are present in the tomato fruits: glucose and fructose and the level of fructose is reported to be slightly higher than that of glucose. In addition 0.1% sucrose has been reported in tomato fruits (Petro-Turza, 1987).

Ascorbate eliminates reactive oxygen species (ROS) through multiple mechanisms (Conklin, 2001). The capacity of ascorbate to directly eliminate several different ROS including $^1O_2$, $O_2^{\cdot -}$ and •OH make it an important component in protection against oxidative stress (Conklin & Barth, 2004; Foyer & Noctor, 2005; Padh, 1990). It also maintains the membrane-bound antioxidant α-tocopherol in the reduced state and indirectly eliminates H_2O_2 through the activity of APX (Asada, 1994), participates in cell metabolism and growth control (Navas & Gomez-Diaz, 1995), cell division (Kerk & Feldman, 1995), expansion of the cell walls (Takahama & Oniki, 1994) and organogenesis (Joy, Patel, & Thorpe, 1998). The two most important carotenoids in fruits of tomato are lycopene, that imparts red color to the tomato fruits and β-carotene, which

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accounts for approximately 7% of the tomato carotenoids (Bilton, Gerber, Grolier, & Leoni, 2001). According to Toor and Savage (2005) tomatoes are a major source of lycopene and carotenoids that have a remarkable antioxidant capacity and eliminate ROS.

Total vitamin C, carotenoids and lycopene therefore, account for the high antioxidant capacity in both fresh and processed tomatoes (Gahler, Otto, & Böhm, 2003), associating the fruit with lower rates of certain types of cancer and cardiovascular diseases (Basu & Imrhan, 2006; Rao & Agarwall, 2000).

Tomato *cv.* H-86 is a commercially prominent cultivar in Uttar Pradesh and eastern part of India and its growth and productivity is influenced under abiotic stresses including drought, heat and salinity (Rai, Singh, & Shah, 2012a). Therefore, we developed the *BcZAT12*-transformed (ZT) transgenic tomato plants that could withstand drought stress earlier in our lab (Rai et al., 2012a; Rai, Singh, & Shah, 2013). The *BcZAT12* transgenics were developed employing the *Agrobacterium*-mediated transformation of tomato var. H-86 (Kashi vishesh) employing a binary vector pBinAR harboring a multiple stress tolerant *ZAT12* gene from *Brassica carinata* under the regulatory control of stress-inducible Bclea1a promoter (Rai et al., 2013; Shah, Singh, & Rai, 2013). Drought resistant tomato plants are reported to have increased plant hight and reduced leaf number per plant (Rana & Kalloo, 1989), however no significant changes in morphological characters of transgenic tomato plants or fruits were noted except slight decrease in plant height and biomass upon exposure to drought. The fruits from the transgenic tomato plants however, are likely to raise concern with quality of tomato fruits obtained from them. The present work was carried out with the aim to evaluate the physico-chemical properties, nutritional quality, sugars concentration, vitamin C, phenolic compounds, flavonoids, lycopene and β-carotene in tomato fruits obtained from non-transformed (NT) and *BcZAT12*-transformed (ZT) cv H-86 plants.

2. Materials and methods

2.1. Plant material and stress conditions

Seeds from six transformed tomato lines (*cv.* H-86 var. Kashi Vishesh) harboring *BcZAT12* gene developed earlier in our lab and confirmed for integration of *BcZAT12* gene in tomato plants were sown and raised inside a net house (Rai, Singh, & Shah, 2012b; Rai et al., 2013). Seedlings of all the six lines were tested by kanamycin spray for the selection of true transgenic plants and raised to ten leaves stage in a net house. The drought treatments began at 10 leaf stage, NT and T tomato plants were subjected to water withdrawal for 21 days. After that plants were maintained till fruit maturity. The non-exposed NT and T plants were taken as control (Rai et al., 2012b, 2013). The control plants received 100% irrigation at field capacity (the amount of water which can be retained by the soil in the pot). Five fruits per plants from non-transformed (NT) and *BcZAT12*-transformed (ZT) tomato lines ZT1-ZT6 were collected at commercial maturity as indicated by red color, stored at 10 °C and evaluated for nutritional quality. All the estimations were carried out in triplicate.

2.2. Physico-chemical characterization of tomato fruits from BcZAT12-transformed and non-transformed tomato

2.2.1. Fruit size

To determine the fruit size the equatorial and longitudinal diameter of five intact mature tomato from NT and ZT plants were taken using digital Vernier Calipers (Fisher Scientific, USA).

2.2.2. pH

Five fruits were chopped, crushed and filtered through a 2 mm diameter steel sieve. The pH values were measured in the just filtered tomato juice according to the method given in AOAC (1990) by using Digital portable pH meter (Eutech Instrument Pvt. Ltd, OAKlon muti-Parameter PCSTestr™ 35, Ayer Rajah Crescent, Singapore).

2.2.3. Total soluble solids

Total soluble solid (TSS) content was also determined in the tomato juice obtained above by using a digital refractometer (ATAGO Pocket Refractometer PAL-1, Tokyo, Japan) with a range of 0–53 °Brix, and a resolution of 0.1 °Brix.

2.3. Measurement of total sugar in fruits from BcZAT12-transformed and non-transformed tomato

The total sugar content in tomato fruit samples from NT and ZT tomato plants were measured according to DuBois, Gilles, Hamilton, Rebers, and Smith (2002). About 200 mg fruit sample was homogenized in 5 ml of 2.5 N/l HCl (Merck, Mumbai, India). The homogenate was centrifuged at 7000 × *g* for 10 min. To 0.5 ml of the supernatant, 0.5 ml of 5 g/100 ml phenol (RFCL, New Delhi, India) was mixed followed by addition of 1 ml of concentrated H_2SO_4. The mixture was left to stand for 10 min and thereafter incubated in a water bath at 30 °C for 20 min. The intensity of the yellow color developed was measured at 490 nm using a spectrophotometer (ELICO SL-159, Sanathnagar, Hyderabad, India). The amount of total sugar is expressed as μg g^{-1} fresh weight.

2.4. Total phenolic content assay in fruits from BcZAT12-transformed and non-transformed tomato

Total phenolics were analyzed with some modification using the Folin–Ciocalteau reagent (Singleton & Rossi, 1965). Each sample (200 mg fruit extract) was extracted with 10 ml methanol (Himedia, Mumbai, India) for 2 min and centrifuged at 19,000 × g for 20 min at 4 °C. 200 μl of methanolic extract (ME) was diluted with 5 ml of distilled water in a test tube. The diluted extract was mixed with 0.5 ml of Folin–Ciocalteau reagent (MP Biomedicals, LLC, Iiikirch, France) and allowed to stand for 3 min. 3.5 ml of 10 g/100 ml sodium carbonate (Merck, Mumbai, India) solution was added to the test tube and the final volume was made up to 10 ml with deionized water. The mixture was placed in a water bath (45 °C for 15 min) and allowed to stand and cool at room temperature. The absorbance was measured at 760 nm in a UV/Vis Nano spectrophotometer (Implen, Southened on sea Essex, UK). The linear reading of the standard curve was from 10 to 100 mg of gallic acid per liter. Total phenolics of tomato fruit is expressed as μg gallic acid equivalent (GAE) g^{-1} fresh weight.

2.5. Estimation of total flavonoids in fruits from BcZAT12-transformed and non-transformed tomato

Total flavonoids content of the tomato extracts (same as in Section 2.2.2) were determined using a modified colorimetric method described previously (Zhishen, Mengcheng, & Jianming, 1999). Both the above extracted methanolic extract (1 ml) and (+)-catechin standard solutions were mixed respectively with 4 ml distilled water and 300 μl of 5 g/100 ml $NaNO_2$ solution, then allowed to mix for 5 min. After that, 300 μl of 10 g/100 ml $AlCl_3$ solution was added and mixed for 1 min. The further 2 ml of 1 mol/l NaOH (Himedia, Mumbai, India) was added and the total volume was made up to 2.4 ml with deionized water. Sample absorbance was read at 510 nm against a prepared blank in a Nano UV/vis