IMPROVEMENT OF SOME NUTRITIONAL VALUES OF TOMATOES *VIA* SALINITY

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Tomato (*Solanum lycopersicon*) is commonly used in the Mediterranean diet and is one of the most widely cultivated economically important vegetable in the world. From the nutritional and health points of view, tomato is characterized by its high content of antioxidants such as vitamin C and vitamin E (Sgherri *et al*., 2007). The consumption of fresh tomatoes and tomato products has been found to be inversely related to the incidence of some types of cancer and cardiovascular diseases (La Vecchia, 1997; Giovannucci, 1999).

Viruses form major threat to tomato plants. The most important virus affecting tomato is Tomato yellow leaf curl virus (TYLCV). Goodman (1977) reported that TYLCV is a member of family Geminivirdae which is transmitted by whiteflies. Morris *et al*. (2002) stated that the detection of TYLCV in tomato plants was achieved two weeks after whitefly fumigation with an improved frequency of detection at four weeks. They also found out that the PCR method is more sensitive than triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) for the detection of TYLCV isolates in all known hosts. Abou-Jawdah *et al*. (2006) used the PCR reaction to confirm the presence of TYLCV in tomato plants in Lebanon. El-Gaied and El-Sherif (2012) stated that some antioxidant activity increased in the TYLCV infected tomato plants.

Soil salinity constitutes a major abiotic stress in plants worldwide. This has led to research involving salt tolerance and isolation of salt induced proteins with the aim of improving crop plants (Hoyos and Zhang, 2000; Majoul *et al*., 2000). Changes in the environmental conditions, such as salt concentration, affect the concentration of vitamins and soluble sugars of cherry tomato (Cristina *et al*., 2008). Tomato, a moderate-salinity-tolerant species, has been shown to respond positively to irrigation with diluted seawater, increasing its nutritional value (Giovannucci, 1999; Cristina *et al*., 2008).

Antioxidants play a fundamental role in the protection of plant cells from oxidative damage. In fact, antioxidants like vitamin C, vitamin E and β-carotene, with their antagonistic functions against free radicals, are very useful to protect humans against various diseases (De Pascale *et al*., 2001). The antioxidant ac-

tivity status is important to determine the nutritional value of fruits and vegetables (Rice-Evans *et al*., 1996). Increased antioxidant levels not only have high benefits in preventing widespread human diseases, including cancer and cardiovascular pathogens but enhance shelf life as well (Sgherri *et al*., 2007). Nutritional value of food products is of great importance for modern consumer society and can be affected by various substances; some of them are antioxidants (Smidova and Izzo, 2009).

Vitamin C (ascorbic acid) is a water soluble organic compound; mammals are not able to synthesize it. Vitamin C reduces hydrogen peroxide (H_2O_2), which preserves cells against reactive oxygen species (Zbynek *et al.*, 2008). Vitamin C plays an important role in resistance to oxidative stresses such as heavy metals, salts, ultraviolet radiation, etc. (Taqi *et al.*, 2011). Vitamin C is important in forming collagen, muscle and blood vessels. It also has rich contents of phytonutrients (Hsu *et al.*, 2008).

Vitamin E applies to a family of eight related compounds, each with its own biological activity, potency and functional use in the body; α-tochopherol is considered as the most powerful antioxidant where it appears to provide better protection than other types of tochopherols (Sgherri *et al*., 2007).

Vitamin E (tocopherols) synthesized exclusively by photosynthetic organisms is a major antioxidant in biomembranes (Ouyang, 2011). Previous studies have shown that stress activated the expression of genes responsible for the synthesis of tocopherols in higher plants (Shao, 2007). Vitamin E and vitamin C are essential components of the human diet and perform numerous critical functions including scavenging and quenching of free radicals and various reactive oxygen species (ROS) (Szymańska and Kruk, 2007).

The present study focused on the changes in some antioxidants, as well as some types of carbohydrates in TYLCV-free tomato plants stressed by using 100 mM NaCl under greenhouse conditions. In particular, vitamin C, vitamin E, glucose and fructose which play a significant role in determining the nutritional value of tomatoes, were analyzed.

MATERIALS AND METHODS

Plant material

Seeds of tomato (Castle Rock) from Agriculture Research Center (ARC) were germinated in greenhouse at AGERI, ARC with 16 hr illumination per day. Plants were kept in cages to avoid infection with TYLCV by whiteflies.

Salt treatment

Sodium chloride was added in concentrations of 100 mM to plants when they were 30 days old. The plants were then grown for the next 45 days during which the salinized solutions were changed every 3 days under greenhouse conditions. Five plants were grown for

salinity treatment and the control plants were irrigated with water. After 45 days of salt treatment, at age of 75 days old, plants were tested for the presence of TYLCV using PCR to confirm that it is free virus, followed by determination of some antioxidants levels as vitamin C, vitamin E using HPLC and glucose and fructose levels using UPLC.

DNA extraction

Leaves of stressed plants, at the age of 75 days old, were used to extract DNA with DNeasy Plant Mini Kit supplied from Qiagen Hilden Germany catalog number 69104 (Riha *et al.*, 1998). Extracted DNA was subjected to PCR using whitefly transmitting geminivirus (WTGs) specific primers HD1 & HD2 and TYLCV-CP specific primers to confirm that the plants are virus-free.

Polymerase chain reaction (PCR)

Primers used in this work were designed from the nucleotide sequence for Tomato yellow leaf curl virus Egyptian isolate (TYLCV-Eg) (Abdallah *et al*., 1993). The oligonucleotide primers were synthesized at Agriculture Genetic Engineering Research Institute, Agriculture Research Center (AGERI, ARC, Giza, Egypt) on an ABI 392 DNA/RNA synthesizer (Applied BioSystem, Lincoln Center Drive, Foster City, CA, USA). The PCR was carried out as described by Essam *et al*. (2004). The primers used to amplify TYLCV-Eg viral genome and position of the PCR products are shown in Table (1).

Vitamins determination

Sample preparation

Five gram of stressed tomato leaves were dried at 105°C till the weight was constant for moisture percent determination. Levels of vitamin C and vitamin E were determined in 75 days old stressed and virus free tomato leaves by a reverse-phase HPLC technique.

Vitamin C level was determined from 1 gm fresh weight stressed tomato leaves as a block. The method was carried out according to Zbynek *et al*. (2008). Twenty µl of supernatant were injected in HPLC (Hewlett Packards series 1050, detector UV 1050 and column ODS 5x4.6x25 ultra sphere).

α-tochopherols were determined in lipid extracts of one gm fresh weight stressed tomato leaves as a block and a control sample. Extraction was performed in the dark according to Nielsen and Hansen (2008) using HPLC as previously indicated.

Glucose and fructose

0.1 gm of dry weight from stressed tomato leaves as a block was analyzed using Acquity ultra performance liquid chromatography (UPLC) H-class waters USA. The detector used was MS/MS detector XEVO-TQD waters USA. The column used was Acquity UPLC. BEH amide 1.7 µm 1.0x100 mm column waters USA. The way of determination was used according to the application note of the instrument.

RESULTS AND DISCUSSION

Seeds of tomato were germinated in greenhouse at AGERI in cages with 16 hr illumination per day. One month old plants were subjected to 100mM NaCl salt stress for 45 days.

Salt level (100 mM NaCl) was studied on tomato plants where caution in practice of over irrigation with salty water should be held to avoid deleterious impact on the soil. Some researchers studied the effect of this concentration on tomato plants (Amir Nawaz *et al.*, 2012). Also, El-Gaied and El-Sherief (2012) found that the concentration of 100 mM NaCl gave the best concentration of antioxidants. This concentration also is in conformity with Syed *et al.* (2011) who studied the effect of 75 mM NaCl salinity on tomato to determine Na^+/K^+ ratio and proline content and with Shah *et al.* (1994) who studied the effect of 30, 60 and 100 mM NaCl on the contents of glucose, fructose, ascorbic acid and citric acid in tomato plants and found that the concentration of 100 mM NaCl gave the best results of glucose and fructose contents.

DNA extraction and PCR

DNA extracted from stressed tomato plants, 75 days old, was used for PCR analysis with WTG specific primers (Cp-F, Cp-R and HD1, HD2). The cloned TYLCV genome was used as positive control in PCR experiments lanes 3 and 6. The DNA extracted from salt-treated plants gave negative results compared with DNA extracted from positive control where a 787 bp band was amplified in case of Cp-F, Cp-R and 674 bp bands was amplified in case of HD1 & HD2 (Fig. 1).

This experiment was carried out to confirm that the plants are virus-free since the TYLCV virus was shown to affect the quantity of antioxidants in tomato plants (El-Gaied and El-Sherif, 2012). This result indicates that the plants are not infected with TYLCV virus as compared with the positive control. These results were in agreement with the results of Abou-Jawdah *et al.* (2006) and Morris *et al.* (2002), as they used the PCR reaction to confirm the presence of TYLCV in tomato plants.

Vitamin quantification

Vitamin quantification was calculated from the curve generated by plotting the peak area of each authentic standard versus concentration (Fig. 2).

Treatment of virus-free tomato plants with 100 mM NaCl increased the contents of vitamin C and Vitamin E (α-tochopherol) by considerable amounts, as compared with the control plants (treated with water). These results represent the average ratio between the plants. These results agreed with Shah *et al.* (1994) who observed considerable enhancement of vitamin C in tomato plants treated with 100 mM NaCl as compared with the control.

Results obtained in this work are also consistent with previous studies of Sgherri *et al.* (2007) and Cristina *et al.*

(2008), who showed the benefit of irrigation with salt solution or diluted sea water on food quality of tomato berries, as salinity increased the amounts of vitamin C and α-tochopherol. They reported that vitamin C could have been involved in the regeneration of α-tochopherol. Similar observations were reported by some investigators (Flavia and Riccardo, 2008). Abbasi *et al*. (2007) who observed that tobacco plants treated with 400 mM NaCl displayed 6 fold increases in total tochopherol content. The total vitamin E content is closely related to concentration of NaCl in the growing medium. Also, Smidova and Izzo (2009) found that elevating salinity level corresponded to electric conductivity of 8 mS/cm of tomato berries cultivar Gimar increased the content of vitamin C and of α-tochopherol. On the other hand, Gossett *et al*. (1996) observed increased amount of α-tochopherol in salt tolerant cotton plants.

Glucose and fructose content

Sugar analysis was calculated from the curve generated by plotting the peak area of each authentic standard versus concentration in Fig. (3).

The contents of glucose in virus-free plants were clearly increased by using 100 mM NaCl salt stress as compared with the control plants while there was a slight increase in the amount of fructose in salt stressed plants compared with the control plants. These results agreed with the results obtained by Amini and Ehsanpour (2005) who found that in response to increasing salt concentration of the medium, the average amount of total carbohydrate in stem-leaf of tomato plants cv. Shirazy increased. Whereas, Adams (1988) reported that increasing the levels of NaCl always improved tomato fruit quality; increased dry matter, sugar contents and acidity of the fruit juices.

The results also agreed with Smidova and Izzo (2009) who reported increasing contents of reducing sugar by 54% in tomato plants cultivar Jama under elevated salinity level.

Change in soluble sugars content under salt stress has already been reported for a number of plant species. Ashraf and Tufai (1995) determined the total soluble sugars content in five sunflower accessions differing in salt stress. They found that sugar content increased in all five lines with increasing salt in the growth medium.

SUMMARY

The antioxidant activity status is important to determine the nutritional value of fruits and vegetables. Increased antioxidant levels not only have high benefits in preventing widespread human diseases, including cancer and cardiovascular pathogens but also it enhances shelf life as well.

This study was carried out to test the effects of 100 mM NaCl on some antioxidants (vitamins C and E) and some monosaccharides (glucose and fructose) of TYLCV virus-free tomato plants (*Solanum lycopersicon*) cultivar Castle Rock.

The plants were treated at 30 days old with 100 mM NaCl for the next 45 days.

At 75 days old, mature leaves were harvested and tested for TYLCV infection, as this virus affects the amount of antioxidants in tomato plants. Plants were kept in cages to avoid infection with TYLCV. The plants gave negative results, confirming that they are virus-free.

Levels of vitamin C and vitamin E were determined by a reverse-phase HPLC technique while levels of glucose and fructose were determined using UPLC-MS in 75 days treated tomato leaves. Plants showed an increase in vitamin C, α-tochopherol, glucose and a slight increase in fructose contents versus the control plants.

These results are of great importance from the nutritional and health points of view where salt stress improved the plant quality by increasing the concentrations of important antioxidants (vitamin C and vitamin E). In conclusion, the use of controlled salinity level can be an effective method to produce tomatoes of good nutritional quality and with higher market price.

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Table (1): Nucleotide sequences of the whitefly transmitting geminiviruses (WTG) specific primers used to amplify the TYLCV-Eg viral genome and position of PCR product.

Primer name	Nucleotides Sequence	Added restriction sites	Position of amplified fragment	Length bp
Cp-F	5'-CGGAATTCACATGTCGAAGCGACCAGG-3'	*Eco*RI	467-1253	787
Cp-R1	5'-CGGGATCCTTAATTTGATAATGAATC-3'	*Bam*HI		
HD-1	5´-CGGAATTCGCCCACCAATAACTGTAGC-3´	*Eco*RI	1855-2528	674
HD-2	5´-CGGGATCCGCAGTCCGTTGAGGAAACTTAC-3´	*Bam*HI		

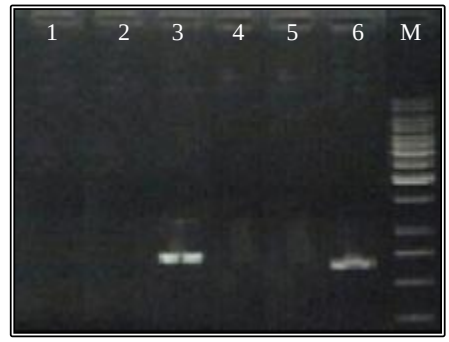

Fig. (1): Amplified PCR fragments produced from using WTGs specific primers (Cp-F & Cp-R and HD1 & HD2). Stressed tomato plants with 100 mM NaCl (lanes 1, 2) showed negative results in case of first couple of primers and lanes (4, 5) showed negative results for second couple of primers compared with the cloned TYLCV genome which was used as positive control (+) lanes (3, 6) and gave the expected molecular weight. Lane M represents the DNA marker (1 KB Ladder from Fermentas).

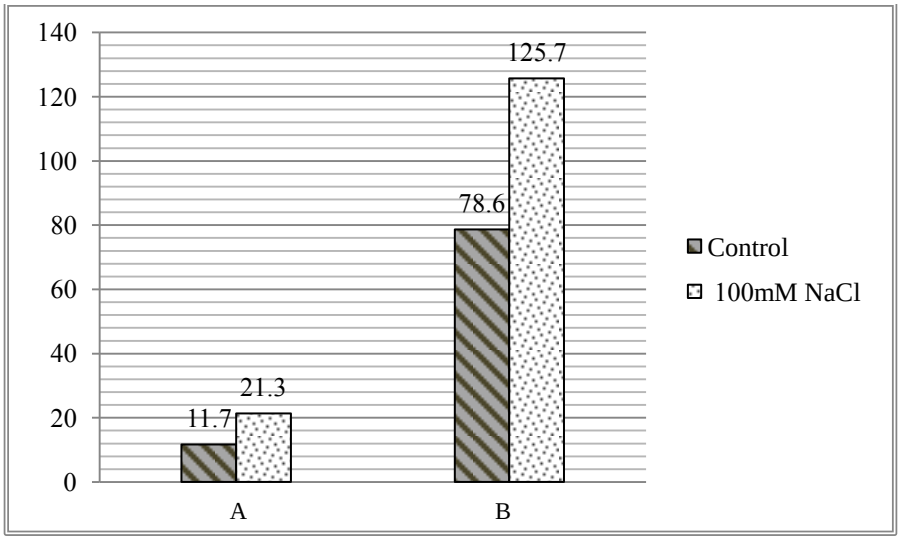

Fig. (2): Effect of 100 mM treatment on average concentration of vitamin C and vitamin E in stressed tomato plants. (A): Concentration of Vitamin C (µg/1000µl) and (B): Concentration of Vitamin E (µg/1000µl).

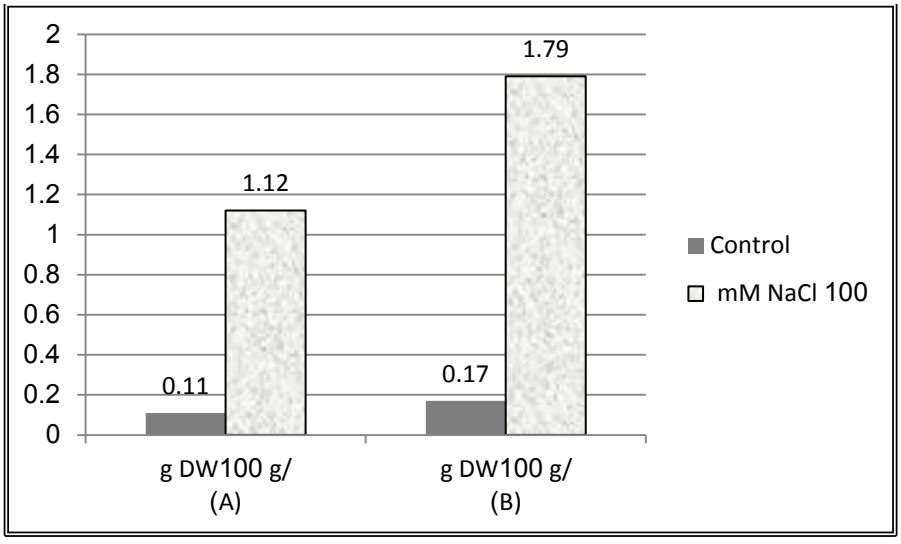

Fig. (3): Effect of 100 mM NaCl treatment on average concentration of (A) fructose% and (B) glucose% in dried leaves of five stressed tomato plants.