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## Tomato fruit quality as influenced by salinity and nitric oxide

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**Abstract:** The impact of salinity (measured by adding 100 mM of NaCl to the nutrient solution) and spraying with sodium nitroprusside (10  $\mu$ M of SNP, NO source) on fruit quality of tomato (Super Strain B) plants grown under field conditions was studied. Irrigation with salinised nutrient solution alone resulted in a significant suppression in fruit fresh and dry biomass, length, diameter, and volume as well as  $\beta$ -carotene and lycopene contents. This decrease was accompanied by a significant increase in Na accumulation, total alkaloids, and antioxidants, including total phenolics and flavonoids, and reduced ascorbic acid (ASA) content. Similar to total phenolics and flavonoids, the content of some individual phenolic acids such as protocatechuic, vanillic, chlorogenic, ferulic, and sinapic acids were at their high levels under saline conditions. Spraying the salinised plants with SNP improved tomato fruit quality to some extent in regards to salinity impact. Under the studied salinity level there was an enhancement of health-promoting compounds (phenolic compounds, flavonoids, and alkaloids) synthesis in tomato fruits, with significant changes in other quality parameters.

**Key words:** *Lycopersicon esculentum*, lycopene,  $\beta$ -carotene, ascorbic acid, phenolic content

### 1. Introduction

Excessive salinity is the most important environmental stress factor that greatly affects the growth, nutrition, and productivity of many plant species (Sayed, 2003). The response of plants to excess salinity is complex and involves morphological and developmental changes as well as physiological and biochemical processes (Khan et al., 2010). Morphologically the most typical symptom of saline injury to plants is the reduction of growth (Jaleel et al., 2008), which is a consequence of several physiological responses including modification of ion balance, water status, mineral nutrition, photosynthetic efficiency, carbon allocation and utilisation, membrane instability, and failure in the maintenance of turgor pressure (Yildirim et al., 2006).

It has been reported that salinity decreases pepper and melon yield (Navarro et al., 2002; Bustan et al., 2005). While excessive salt exposure reduces tomato fruit size, total yield, and photosynthesis and increases blossom end rot (Saito et al., 2006), moderate salt stress generally improves fruit quality by increasing carotenoids and total soluble solids, which are important components of taste in tomatoes (sugars, organic acids, and amino acids) (De Pascale et al., 2001; Krauss et al., 2006).

Plant phenolics have often been referred to as secondary metabolites, and many of these compounds

play an essential role in the regulation of plant growth and development and could be enhanced as powerful antioxidants in plant tissues under different stresses, such as salinity (Dixon and Palva, 1995). Total phenolic content increased with salinity levels in fruits like apple and strawberry (Navarro et al., 2006; Keutgen and Pawelzik, 2008). Recently, Rezazadeh et al. (2012), working with artichoke leaves, concluded that moderate saline induced the saline tolerance pathway via increasing total phenolic and flavonoid compounds.

Nitric oxide (NO) is a bioactive gaseous molecule involved in the signalling process within plants and plays a central role in a variety of physiological processes including germination, senescence, flowering, repining of fruits, and response to biotic and abiotic stresses (Leshem, 2000; Zheng et al., 2010). Wu et al. (2011) reported that NO applied during salt exposure significantly attenuated salt-induced oxidative damage. Some previous work has demonstrated that NO could delay ripening and improve the postharvest quality of tomato (Shaoying et al., 2005) when applied as short-term fumigation at low concentrations. It is suggested that NO might exert a profound influence on fruit by inhibiting ethylene production (Leshem, 2000). Furthermore, NO treatment reduced the degree of disintegration of the cell membrane with less electrolyte leakage, which resulted in better

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retention of the cellular components such as pigments, titratable acidity, soluble solids, and free antioxidant compounds, particularly ascorbic acid, which the fruits are most valued for (Manjunatha et al., 2010).

Tomato is one of the most important horticultural crops in the world. In terms of human consumption and health, it is a major component of daily meals in many countries and constitutes an important source of potassium, vitamins E and C, folic acid, and many health beneficial factors like carotenoids (lycopene and  $\beta$ -carotene) that have been shown to be effective against some cancer cells (Tang et al., 2008). It is also a good source of polyphenolic compounds, such as flavonoids and hydroxycinnamic acids (Bugianesi et al., 2004).

The aim of the present investigation was to study the effect of salt stress on tomato fruit quality and assess the role of NO (applied exogenously as SNP) in the response of tomato fruit to salinity. The changes in some growth parameters, carotenoids ( $\beta$ -carotene and lycopene), vitamin C, and secondary metabolites including individual phenolics compounds, total flavonoids, alkaloids, phenolics, and anthocyanins were followed.

## 2. Materials and methods

### 2.1. Plant material, growth conditions, and treatments

Tomato seeds (*Lycopersicon esculentum* Mill. 'Super Strain B') were obtained from the Agricultural Research Centre, Giza, Egypt. They were surface sterilised with 2.5% sodium hypochlorite for 10 min, rinsed with distilled water, and soaked for 24 h at 25 °C in aerated water. Sodium nitroprusside (SNP) was used as NO donor, and NaCl was used to apply salt stress. To choose suitable concentrations of SNP and NaCl, a preliminary experiment was conducted where seeds were allocated at random in petri dishes (15 cm diameter, 20 seeds per dish) containing filter paper moistened with 20 mL of 0, 5, 10, 20, 50, 75, and 100  $\mu$ M SNP; covered by a lid; and incubated at 27  $\pm$  2 °C for 4 days. Similarly, another group of seeds were tested for different concentrations of NaCl: 0, 10, 20, 50, 75, 100, 150, and 200 mM. The germination percentage was calculated as a standard of radicle emergence, and the specified concentration of SNP and NaCl was determined as 100 mM NaCl and 10  $\mu$ M of SNP.

Seeds of uniform size were sterilised, as previously mentioned, and sown in May in weighed plastic pots (40  $\times$  33 cm, 5 seeds per pot) filled with a fixed amount of clay soil. The pots were irrigated with one-tenth strength Hoagland's solution, and after 30 days the pots were divided into 2 sets; one set (control treatment) was irrigated with one-tenth strength Hoagland's solution and the other with one-tenth strength Hoagland's solution containing 100 mM of NaCl (salt treatment). The experiment was carried out under natural environmental conditions, and the irrigation with

NaCl was performed once weekly. On day 60 (at the fourth or fifth true leaf stage) NO treatment was started, and 100 mL of 10  $\mu$ M SNP solutions were applied once weekly by spraying the leaves of the control and salt stressed plants for 1 month until the plants reached 90 days (flowering stage). The tomato fruits were collected after 120 days, used for measuring the different growth parameters, and kept for all chemical analyses. In summary, the experimental design consisted of 4 treatments: 1) control, 2) SNP spray, 3) salt, and 4) salt + SNP spray and was arranged in a randomised, complete block design with 3 replicates.

### 2.2. Measurements of physiological parameters

#### 2.2.1. Growth parameters

After 120 days the fruit fresh (FW) and dry weights (DW) were determined. The fruit length and diameter were measured as described by Adedeji et al. (2006), while the volume of the fruit was measured using the water displacement method (Rashidi and Seyfi, 2007).

#### 2.2.2. Na content

Sodium concentration was determined by flame photometer (CORN NG 400) following wet digestion of oven dried tissue, as described by Chapman and Pratt (1982).

#### 2.2.3. Total alkaloid content

The total alkaloid contents of tomato fruits were measured using the 1,10-phenanthroline method, as described by Singh et al. (2004). The reaction mixture contained 1 mL of ethanolic extract, 1 mL of 0.025 M FeCl<sub>3</sub> in 0.5 M HCl, and 1 mL of 0.05 M 1,10-phenanthroline in ethanol and was incubated at 70  $\pm$  2 °C. The absorbance was read at 510 nm, and the total alkaloid content was calculated from the standard curve obtained from different concentrations of colchicines and expressed as micrograms per gram FW.

#### 2.2.4. Total flavonoid content

The colorimetric methanolic aluminium chloride method was used for total flavonoid estimation (Luximon-Ramma et al., 2002). The reaction mixture contained 1.5 mL of the acetone plant extract and 1.5 mL of 2% methanolic aluminium chloride, and the absorbance was measured at 367 nm. Total flavonoid contents were calculated with the standard curve of quercetin, and values were expressed micrograms per gram FW.

#### 2.2.5. Total phenolic content

Total phenolic contents of tomato fruits were determined using the modified Folin–Ciocalteu reagent (McDonald et al., 2001). An aliquot of plant extract was added to 1.58 mL of distilled water and 100  $\mu$ L of Folin–Ciocalteu reagent. The reaction mixture was shaken and allowed to stand for 5 min before the addition of 300  $\mu$ L of 20% NaCO<sub>3</sub>. After 20 min at 40 °C, the absorbance was measured at 765 nm against each blank. The content of phenol was calculated from the standard curve obtained from different

concentrations of gallic acid and expressed as milligrams per gram FW.

#### 2.2.6. Anthocyanin content

Anthocyanin was extracted according to the procedure described by Mancinelli et al. (1976). An aliquot of the powdered plant material was extracted with methanol containing 1% (v/v) HCl, and absorption was determined spectrophotometrically at 530 and 657 nm.

#### 2.2.7. Lycopene content

Lycopene was spectrophotometrically estimated according to the method of Fish et al. (2002). Approximately 0.3–0.6 g samples were added to 5 mL of 0.05% (w/v) BHT in acetone, 5 mL of ethanol, and 10 mL of hexane. The recipient was introduced in ice and stirred on a magnetic stirring plate for 15 min. After shaking, 3 mL of deionised water were added, and the samples were shaken for 5 min on ice. Samples were then left at room temperature for 5 min to allow the separation of both phases. The absorbance of the hexane layer (upper layer) was measured at 503 nm blanked with hexane.

#### 2.2.8. High-performance liquid chromatography (HPLC) analysis

##### 2.2.8.1. Ascorbic acid

For estimation of ascorbic acid content (ASA), 1 g of frozen fruit tissues was homogenised in 5 mL of ice-cold 6% *m*-phosphoric acid (pH 2.8) containing 1 mM EDTA (Gossett et al., 1994). The homogenate was centrifuged at  $20,000 \times g$  for 15 min at 4 °C. The supernatant was filtered through a 30- $\mu$ m syringe filter, and 50  $\mu$ L of the filtrate was analyzed using a HPLC system (PerkinElmer series 200 LC and UV/VIS detector 200 LC, USA) equipped with a 5- $\mu$ m column (Spheri-5 RP-18;  $220 \times 4.6$  mm; Brownlee) and UV detection at 245 nm with 1.0 mL/min water (pH 2.2) as the mobile phase, run isocratically (Gahler et al., 2003).

##### 2.2.8.2. $\beta$ -carotene

$\beta$ -carotene was extracted by grinding fruit tissues in a solution of 100% acetone containing  $\text{CaCO}_3$  (Jung, 2004). The extracts were centrifuged at  $16,000 \times g$  for 10 min, and 20  $\mu$ L of the resulting supernatants were used for HPLC analysis, as described by Gilmore and Yamamoto (1991) using the previously mentioned HPLC system. Solvent A (acetonitrile, methanol, Tris-HCl buffer 0.1 M, pH 8.0, 72:8:3) was run isocratically from 0 to 4 min followed by a 2.5 min linear gradient to 100% solvent B (methanol, hexane, 4:1) at a flow rate of 2 mL/min. The detector was set at 440 nm for the integration of peak areas after calibration with the external standard.

##### 2.2.8.3. Individual phenolic compounds

The individual phenolic compounds were extracted in 80% methanol, as described by Szauffer-Hajdrych et al. (2008), and 20  $\mu$ L were immediately injected by analytic sample injector using the same HPLC system described above. The mobile phase consisted of the following linear gradient: 5% methanol, 95% water (pH 2.6) and 80% methanol, 20% water (pH 2.2). The flow rate was 1 mL/min, and the UV detector was set at 290 nm for the integration of peak areas after calibration with the external standard (Garcia-Salas et al., 2010).

##### 2.2.9. Statistical analysis

Each experiment was repeated at least 3 times. Values were expressed as means  $\pm$  standard deviation (SD). The data of all experiments were analyzed using least significant differences (LSD) at a level of  $P \leq 0.05$ , according to Steel and Torrie (1980).

### 3. Results

In the present investigation NaCl significantly decreased fruit fresh and dry biomass, length, diameter, and volume (Table 1). The decrease in the fresh and dry biomass was about 73.5% and 52% of the control values. Exogenous application of SNP shifted off, to some extent, the inhibitory effect of salinity on various growth parameters of tomato fruit (Table 1).

**Table 1.** Effect of exogenously sprayed SNP (10  $\mu$ M) on some quality parameters of tomato fruits (length, diameter, volume, fresh and dry weights) grown under 100 mM NaCl. Values are the means of 3 independent replicates  $\pm$  SD; means followed by different letters are significantly different at  $P \leq 0.05$  according to least significant difference (LSD).

Treatment	Fresh weight g fruit <sup>-1</sup>	Dry weight g fruit <sup>-1</sup>	Fruit length cm	Fruit diameter cm	Fruit volume cm <sup>3</sup>
Hoagland's	26.95 $\pm$ 2.7 <sup>b</sup>	2.12 $\pm$ 0.166 <sup>a</sup>	3.3 $\pm$ 0.285 <sup>b</sup>	3.8 $\pm$ 0.22 <sup>b</sup>	14.5 $\pm$ 1.22 <sup>b</sup>
SNP only	53.52 $\pm$ 4.98 <sup>a</sup>	2.63 $\pm$ 0.207 <sup>a</sup>	5.0 $\pm$ 0.56 <sup>a</sup>	6.0 $\pm$ 0.55 <sup>a</sup>	57.0 $\pm$ 4.85 <sup>a</sup>
Salt only	7.14 $\pm$ 0.81 <sup>c</sup>	1.02 $\pm$ 0.087 <sup>b</sup>	2.2 $\pm$ 0.12 <sup>c</sup>	2.2 $\pm$ 0.16 <sup>b</sup>	5.0 $\pm$ 0.41 <sup>c</sup>
Salt + SNP	20.84 $\pm$ 1.99 <sup>b</sup>	2.2 $\pm$ 0.14 <sup>a</sup>	3.0 $\pm$ 0.22 <sup>b</sup>	3.3 $\pm$ 0.21 <sup>b</sup>	18.0 $\pm$ 0.103 <sup>b</sup>

### 3.1. ASA content

Salt stress in SNP-treated and untreated plants significantly increased ASA content of tomato fruit (1.97- and 7.09-fold, respectively) compared to their controls (Table 2).

### 3.2. Na content

Salt stress also resulted in an increase in the Na content in both SNP-treated and untreated plants. However, spraying salinised tomato with SNP decreased the Na<sup>+</sup> accumulation in fruit tissues by 63.5% compared to salinised plants (Table 2).

### 3.3. Lycopene and $\beta$ -carotene

Under the prevailing experimental conditions, salinity decreased  $\beta$ -carotene and lycopene contents of tomato fruits by about 94% and 64.5%, respectively (Table 2). SNP treatment alone resulted in a significant increase in lycopene content, and that was associated with a significant decrease in  $\beta$ -carotene, while it has almost no effect under salt stress.

### 3.4. Total flavonoid, alkaloid, phenolic, and anthocyanin contents

There was a significant increase in the total phenolic, flavonoid, and alkaloid contents of tomato fruits under

salinity conditions by about 16.5%, 93.5%, and 97.7%, respectively, compared to the control (Table 3). Under the prevailing experimental conditions, salinity decreased the anthocyanin content, while SNP treatment significantly increased it. Data in Table 3 also demonstrate that spraying the salinised tomato plants with SNP resulted in a significant increase in total phenolic content, while the total alkaloids remain almost constant when compared to unsprayed plants.

### 3.5. Individual phenolic compounds

Under the prevailing experimental conditions, salinity increased some individual phenolic acids such as the protocatechuic, vanillic, chlorogenic, ferulic, and sinapic acids of SNP-treated or untreated tomato fruits. Conversely, coumaric and cinnamic acids were markedly decreased (Table 4). These results suggest that salinity enhances the biosynthesis of these acids at the expense of their precursors (cinnamic and coumaric).

For example, chlorogenic acid increased by about 55% in salt stressed tomato fruit, while cinnamic acid decreased by about 23%. SNP had an inductive effect on some phenolic acids, such as ferulic, chlorogenic, and protocatechuic.

**Table 2.** Effect of exogenously sprayed SNP (10  $\mu$ M) on the Na, ASA, lycopene, and  $\beta$ -carotene contents of tomato fruits grown under 100 mM NaCl. Values are the means of 3 independent replicates  $\pm$  SD; means followed by different letters are significantly different at  $P \leq 0.05$  according to least significant difference (LSD).

Treatment	Na content mmol g <sup>-1</sup> FW	ASA $\mu$ g g <sup>-1</sup> FW	Lycopene mg g <sup>-1</sup> FW	$\beta$ -carotene $\mu$ g g <sup>-1</sup> FW
Hoagland's	1.80 $\pm$ 0.109 <sup>b</sup>	11.19 $\pm$ 1.095 <sup>b</sup>	16.91 $\pm$ 1.42 <sup>b</sup>	1.89 $\pm$ 0.109 <sup>a</sup>
SNP only	1.73 $\pm$ 0.098 <sup>b</sup>	23.72 $\pm$ 3.07 <sup>c</sup>	35.57 $\pm$ 3.07 <sup>a</sup>	0.57 $\pm$ 0.032 <sup>b</sup>
Salt only	9.87 $\pm$ 1.06 <sup>a</sup>	79.41 $\pm$ 6.88 <sup>a</sup>	5.99 $\pm$ 0.69 <sup>c</sup>	0.117 $\pm$ 0.01 <sup>c</sup>
Salt + SNP	3.60 $\pm$ 0.36 <sup>c</sup>	46.8 $\pm$ 5.03 <sup>d</sup>	6.89 $\pm$ 0.711 <sup>c</sup>	0.197 $\pm$ 0.011 <sup>c</sup>

**Table 3.** Effect of exogenously sprayed SNP (10  $\mu$ M) on the total phenolic, alkaloid, flavonoid, and anthocyanin contents of tomato fruits grown under 100 mM NaCl. Values are the means of 3 independent replicates  $\pm$  SD; means followed by different letters are significantly different at  $P \leq 0.05$  according to least significant difference (LSD).

Treatment	Total phenolics mg g <sup>-1</sup> FW	Total alkaloids $\mu$ g g <sup>-1</sup> FW	Total flavonoids $\mu$ g g <sup>-1</sup> FW	Anthocyanin mg g <sup>-1</sup> DW
Hoagland's	37.9 $\pm$ 2.96 <sup>b</sup>	4.296 $\pm$ 0.28 <sup>b</sup>	0.062 $\pm$ 0.001 <sup>b</sup>	0.060 $\pm$ 0.013 <sup>b</sup>
SNP only	22.53 $\pm$ 2.07 <sup>b</sup>	3.528 $\pm$ 0.36 <sup>b</sup>	0.036 $\pm$ 0.0025 <sup>b</sup>	0.070 $\pm$ 0.008 <sup>ab</sup>
Salt only	44.166 $\pm$ 3.69 <sup>ab</sup>	8.496 $\pm$ 0.84 <sup>a</sup>	0.120 $\pm$ 0.0085 <sup>a</sup>	0.040 $\pm$ 0.013 <sup>b</sup>
Salt + SNP	51.96 $\pm$ 5.22 <sup>a</sup>	8.244 $\pm$ 0.92 <sup>a</sup>	0.040 $\pm$ 0.013 <sup>b</sup>	0.104 $\pm$ 0.092 <sup>a</sup>

**Table 4.** Effect of exogenously sprayed SNP (10  $\mu\text{M}$ ) on the phenolic composition of tomato fruits grown under 100 mM NaCl using HPLC analysis.

Treatment	Phenolic compounds $\mu\text{g g}^{-1}$ FW								
	Gallic	Protocatechuic	Vanillic	Chlorogenic	Esculetin	Ferulic	Sinapic	Coumaric	Cinnamic
Hoagland's	335	116	144	128	116	100	124	153	132
SNP only	217	146	187	63	162	113	172	106	119
Salt only	184	238	217	199	116	180	257	93	101
Salt + SNP	219	374	234	215	96	235	323	51	46

## 4. Discussion

### 4.1. Growth parameters

In the present study, NaCl significantly decreased fruit fresh and dry biomass and several other growth and quality parameters (Table 1). Similar results were also reported for tomato (İnal, 2002; Rahman et al., 2006; Saeed and Ahmad, 2009) and strawberry (Khayyat et al., 2007) grown in saline soil. In contrast, several authors reported that fruit dry weight significantly increased under saline conditions in a number of horticultural crop species including tomato (Krauss et al., 2006; Gautier et al., 2010) and cucumber (Chartzoulakis, 1992). Exogenous application of SNP has shifted off to some extent the inhibitory effect of salinity on various growth parameters of tomato fruit. This may be explained by the role of SNP in protecting plasma membrane integrity. In a previous work in our lab it was demonstrated that NO could act as an antioxidant signal counteracting arsenic and salt stress in mung bean and tomato plants, respectively, by reducing reactive oxygen species content and enhancing some antioxidant enzyme activities (Ismail, 2012; Amany et al., 2013). Kausar et al. (2013) reported that NO application played a protective role against salt-induced oxidative damage in *Triticum aestivum* by effectively scavenging reactive oxygen species through increased activities of antioxidant enzymes.

### 4.2. ASA content

Under the prevailing experimental conditions, increased concentrations of ASA in tomato fruits grown under saline conditions was in agreement with data reported for other tomato varieties grown under similar conditions (De Pascale et al., 2001; Dumas et al., 2003; Dorais et al., 2008). In contrast, Fanasca et al. (2007) recorded a decrease in ASA content of tomato fruits grown under salinity. Navarro et al. (2006) reported also that salinity decreased the ASA content of pepper fruits, and this effect was dependent on the maturity stage. However, the contradictory results reported on the impact of salinity on ascorbic acid content in tomato fruit might be related to genetic differences in sensitivity to salinity stress, differences in the intensity of

salinity applied to the plant, and interactions with other factors like ripening stage (Dumas et al., 2003). In addition, the possibility for a plant to limit salt accumulation within its tissues triggers differences in the intensity of salinity stress perceived by the plant. Furthermore, it is well known that ascorbic acid is an important component of several fruits (tomato, pepper, and strawberry) that reacts with singlet oxygen and other free radicals and suppresses peroxidation (Dorais et al., 2008).

### 4.3. Na content

Salt stress significantly increased the sodium content of tomato fruit (Table 2). In agreement with these data, several authors reported that salt stress induced the accumulation of  $\text{Na}^+$  in tomato fruit, and this may also result in an enhancement of oxidative parameters (Gautier et al., 2010). Thus, the increased ASA content recorded under salt stress might be linked to the key role of ascorbic acid as a non-enzymatic system and a strong antioxidant in response to the salinity-induced oxidative damage. Spraying salinised tomato with SNP decreased the  $\text{Na}^+$  accumulation in fruit tissues, and this indicates that SNP may protect plasma membrane integrity against the lipo-oxygenative processes.

### 4.4. Lycopene and $\beta$ -carotene

Among several horticultural crops, tomato has been reported to be the predominant source of carotenoids, which play an important role in fruit colouring (Dorais et al., 2008). In addition, lycopene and  $\beta$ -carotene are widely known as powerful natural antioxidants that act as the most efficient singlet oxygen quenchers in vitro among common carotenoids (Di Mascio et al., 1989). In the present study, salinity decreased  $\beta$ -carotene and lycopene contents of tomato fruits (Table 2). In agreement with these data, Dorais et al. (2000) showed that  $\beta$ -carotene in tomato fruit was significantly decreased under salt stress. Riggi et al. (2008) found that water stress had a negative effect on lycopene accumulation during tomato ripening but had no effect on  $\beta$ -carotene.

In contrast, Krauss et al. (2006) reported that moderate salinity enhances lycopene and  $\beta$ -carotene in fresh tomato fruit, although this was not confirmed by the results of Fernández-García et al. (2004). However, according to de Pascale et al. (2001), the total carotenoid and lycopene concentrations in tomato fruit are enhanced by moderate salinity but decrease as the level of salinity exceeds a threshold value.

Carotenoids are intimately linked with photosynthesis as a part of the light harvesting system, and it is well known that salinity suppresses photosynthesis (Chartzoulakis and Klapaki, 2000). Thus, under the prevailing experimental conditions the decrease in lycopene and  $\beta$ -carotene contents may relate to the decrease in photosynthetic processes under salinity. A possible explanation would be that salinity may inhibit or upregulate the biosynthetic pathway of carotenoids via inhibition of the genes encoding enzymes related to lycopene and  $\beta$ -carotene (Dumas et al., 2003). Recently, Babu et al. (2011) reported that salt stress caused an inhibition in the expression of the gene encoded for lycopene  $\beta$ -cyclase, the enzyme that converts lycopene to beta carotene.

SNP treatment alone resulted in a significant increase in lycopene content, and that was associated with a significant decrease in  $\beta$ -carotene; it has almost no effect under salt stress. Therefore, SNP treatment alone may block the enzymatic activities of  $\beta$ -carotene biosynthesis (e.g.,  $\beta$ -cyclase) and, consequently, enhances the synthesis of other antioxidant components such as lycopene that protect the plants against the generation of oxidative chain. However, further studies are necessary to confirm this view.

#### 4.5. Total flavonoid, alkaloid, phenolic, and anthocyanin contents

The results on phenol contents are in conformity with the findings in pepper (Navarro et al., 2006) and tomato fruits (Krauss et al., 2006), while it contrasts with those of Maggio et al. (2007) in other tomato varieties. In addition, Shi et al. (2002) reported that adding NaCl to the nutrient solution did not affect phytonutrients such as flavonoids (quercetin).

It is well known that anthocyanins are members of the flavonoid class of plant secondary metabolites that are not usually synthesised in tomato fruits (Mes et al., 2008). In the present investigation, salinity had almost no effect on anthocyanin content, while SNP treatment significantly increased it. Ganjewala et al. (2008) reported that SNP treatment increased the levels of anthocyanin and flavonol glycosides in pea leaves, most probably via its inhibitory effects on photosynthesis.

The increased synthesis of total phenolic, flavonoid, and alkaloid contents under saline conditions may reflect some kind of defence against stress conditions (i.e. oxidative

burden) since salt stress was accompanied by increased production of reactive oxygen species (Rezazadeh et al., 2012).

Spraying the salinised tomato plants with SNP resulted in a significant increase in total phenolic contents, while total alkaloids remain almost constant compared to unsprayed plants. These observations reveal that the bioactive molecule NO (as SNP) may be an inducer for the biosynthesis of secondary metabolites (phenolics and anthocyanin) which act as oxygen scavengers to reduce oxidative stress and, hence, increase the growth and maturity of tomato fruits (Table 3).

#### 4.6. Individual phenolic compounds

It has been reported that environmental stresses such as salinity lead to the accumulation of polyphenol constituents (Dixon and Palva, 1995). In the present study, salinity resulted in modulating several phenolic acids. For example, chlorogenic acid increased while cinnamic acid decreased in salt stressed tomato fruit. These results were in accordance with the results in several other plants such as artichoke leaves and tomato fruits (Sgherri et al., 2007; Rezazadeh et al., 2012) grown under saline conditions. Furthermore, several types of wounding of apple fruits and leaves induce accumulation of chlorogenic acid and flavanols via activating PAL (Michalek et al., 1999).

The results of the present study also suggest that salinity enhances the biosynthesis of these acids as salt-stress-induced components that could play an important role in diminishing the oxidative processes. These results support the theory that polyphenols as secondary metabolites protect plant tissues against oxidative stress generated by salinity and contribute to salinity tolerance. The phenolic compounds of fruit may contribute to antioxidant intake, which is presumed to have a health-protective action (Kroon et al., 1999). For example, recent research indicated that benzoic and cinnamic acid derivatives have been recognised as potent antioxidants (Natella et al., 1999). In addition, Sgherri et al. (2007) reported that chlorogenic and caffeic acid can act as antioxidants due to their polyhydroxy nature.

As shown in Table 4, the induction effect of SNP on the increase in some phenolic acids under salt conditions may confirm the hypothesis that NO can act as an inducer for biosynthesis of secondary metabolites (total phenolics and anthocyanin), which act as oxygen scavengers to reduce oxidative stress.

Application of NO could improve tomato fruit quality in the face of salinity by enhancing health-promoting compound (phenolic compounds, flavonoids, and alkaloids) synthesis in tomato fruits along with significant changes in other quality parameters.

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