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

Hala Ezzat Mohamed<sup>1</sup> and Ghada Saber M. Ismail<sup>2</sup>

<sup>1,2</sup>Botany Department, Faculty of Science, Alexandria University, 21511, Alexandria, Egypt.

#### ABSTRACT

In the present investigation, the impact of salinity (by adding 100 mM NaCl to the nutrient solution as well as spraying with sodium nitroprusside, 10 µM SNP, NO source) on the fruit quality of tomato (Super strain B) plants grown under field conditions were studied. Irrigation with salinized nutrient solutions alone resulted in a significant suppression in the fruit fresh and dry biomass, length, diameter and volume as well as -carotene and lycopene contents. This decrease was accompanied with a significant increase of Na accumulation, total alkaloids and antioxidants including total phenolics, flavonoids and ascorbic acid (ASA) contents. Similar to total phenolics and flavonoids, the content of some individual phenolic acids such as protocatechuic, vanillic, chlorogenic, ferulic and sinapic acids were of their high levels under saline conditions. Spraying the salinized plants with SNP improved the tomato fruit quality, to some extent, from salinity impact. Under the studied salinity level there was an enhancement in the synthesis of health-promoting compounds (phenolic compounds, flavonoids, ASA and alkaloids) in tomato fruits, with significant positive changes of other quality parameters. Key words: Tomato fruit; Salinity; Nitric oxide; Lycopene; -carotene; Ascorbic acid; Phenolic content

#### **INTRODUCTION**

Excessive salinity is the most important environmental stress factor that greatly affects the growth, nutrition and productivity of many plant species (Munns, 2002; Kao et al., 2003; Sayed, 2003). The response of plants to excess salinity is complex and involves morphological and developmental changes as well as physiological and biochemical processes (Hasegawa et al., 2000; Siddiqui et al., 2009; Khan et al., 2010). Morphologically the most typical symptom of saline injury to plant is the reduction of growth (Azooz et al., 2004; 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 utilization, membrane instability and failure in the maintenance of turgor pressure (Muranaka et al., 2002; Murphy and Durako, 2003; Taylor et al., 2004; Yildirim et al., 2006).

It has been reported that salinity decreases pepper, cucumber, and melon yield (Navarro et al., 2002; Bustan et al., 2005; Tiwari et al., 2010). While excessive salt exposure reduces tomato fruit size, total yield and photosynthesis and increases blossom end rot (Gao et al., 1998; Bolarin et al., 2001; 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) (Balibrea et al., 2000; De Pascale et al., 2001; Krauss et al., 2006; Saito et al., 2008).

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 a powerful antioxidant in plant tissues under different stresses such as salinity (Dixon and Palva, 1995; Parida et al., 2004).

Nitric oxide (NO) is a bioactive gaseous molecule involved in signalling process within plants (Wendehenne et al., 2004) and plays a central role in a variety of physiological processes including germination, flowering, ripening of fruits and response to biotic and abiotic stresses (Romero-Puertas et al., 2004, Zheng et al., 2010). Nasibi and Kalantari (2009) and Wu et al (2011) reported that NO applied during salt exposure, significantly attenuated the salt-induced oxidative damage.

Tomato is one of the most important horticultural crops of 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, vitamin E and C, folic acid and many health beneficial factors like carotenoids (lycopene and carotene) that have been shown to be effective against some cancer cells (Erhardt et al., 2003; Tang et al., 2008). It is also a good source of polyphenolic compounds, such as flavonoids and hydroxycinnamic acids (Martínez-Valverde et al., 2002; Bugianesi et al., 2004). The objective of the present investigation was to study the effect of salt stress on the tomato fruit quality and to access the role of NO (applied exogenously as SNP) on the response of tomato fruit to salinity. The changes in some growth parameters, carotenoids ( carotene and lycopene), vitamin C and secondary metabolites including individual phenolics compounds, total flavonoids, alkaloids, phenolics and anthocyanins were followed.

It has been reported that total phenolic content increased with salinity level in various fruits like pepper 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.

<sup>\*</sup> Corresponding Author: halaamzn@yahoo.com

#### MATERIALS AND METHODS

#### Plant Material, growth conditions and treatments:

Tomato seeds (Lycopersicon esculentum Mill. obtained Super Strain B) were from the Agricultural Research Center, Giza, Egypt. They were surface sterilized 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 to apply salt stress. To choose the suitable concentration of SNP and NaCl, preliminary experiments were 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 µM SNP, covered by lid, and incubated at 27±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 radical emergence and the specified concentration of SNP and NaCl was found after which the germination percentage dropped to about half and was 100 mM NaCl and 10  $\mu$ M SNP.

Seeds of uniform size were sterilized as previously mentioned and sown on May in weighed plastic pots (40×33 cm, 5 seeds per pot) filled with fixed amount of clay soil. The pots were irrigated with 1/10 strength Hoagland solution and after 30 days, the pots were divided into two sets, One set (control treatment) was irrigated with 1/10 strength Hoagland solution and the other with 1/10 strength Hoagland solution containing 100 mM NaCl (salt treatment). The experiment was carried out under natural environmental conditions and the irrigation with NaCl was performed once weekly. On the day 60 (at the fourth or fifth-true leaf stage), NO treatment was started. 100 ml of 10  $\mu M$  SNP solutions were applied once weekly by spraying the leaves of control and salt stressed plants for one month till the plants reached 90 days (flowering stage). The tomato fruits were collected after 120 days and were used for measuring the different growth parameters and were kept for all chemical analyses. In summary, the experimental design consisted of four treatments, 1) control, 2) SNP spray, 3) salt and 4) salt + SNP spray and was arranged in a randomized, complete block design with three replicates.

#### Measurements of physiological parameters:

#### Growth parameters

After 120 days the fruit fresh weights (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).

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) and was expressed as m mol  $g^{-1}$  FW.

Total Alkaloid content

The total alkaloid contents of tomato fruits were measured using 1,10-phenanthroline method as described by Singh *et al.*, (2004). The reaction mixture contained 1 ml ethanolic extract, 1 ml of 0.025 M FeCl<sub>3</sub> in 0.5 M HCl and 1 ml of 0.05 M of 1,10-phenanthroline in ethanol and was incubated at 70±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 colchicine and expressed as  $\mu$ g g<sup>-1</sup> FW.

Total flavonoid content

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 2% methanolic aluminum chloride and the absorbance was measured at 367 nm. Total flavonoids contents were calculated with help of standard curve of quercetin and values were expressed  $\mu$ g g<sup>-1</sup> FW.

Total phenolic content

Total phenolic contents of tomato fruits were determined using the modified Folin-Ciocalteau reagent (McDonald *et al.*, 2001). An aliquot of plant extract was added to 1.58 ml dist. water and 100  $\mu$ L of Folin- Ciocalteau reagent. The reaction mixture was shaken and allowed to stand for 5 min., before 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 concentration of gallic acid and expressed as mg g<sup>-1</sup> FW.

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.

#### Lycopene content

Lycopene was spectrophotometrically estimated according to the method of Fish *et al.*, (2002). Approximately 0.3 to 0.6 g samples were added to 5 ml of 0.05% (w/v) Butylated hydroxyltoluene (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 deionized 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.

#### High-Performance Liquid Chromatography (HPLC) Analysis of:

#### Ascorbate

For estimation of ascorbate content (ASA), one g of frozen fruit tissues was homogenized 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 µm syringe filter and 50 µl of the filtrate were analyzed using a HPLC system (Perkin Elmer series 200 LC and UV/VIS detector 200 LC, USA) equipped with a 5 µm column (Spheri-5 RP-18, 220 × 4.6 mm, Brownlee) and UV detection at 245 nm with 1.0 ml/min water (pH 2.2) as the mobile phase, run isocracticaly (Gahler *et al.*, 2003).

#### -carotene

-carotene was extracted by grinding fruit tissues in a solution of 100% acetone containing CaCO<sub>3</sub> (jung, 2004). The extracts were centrifuged at  $16,000 \times g$  for 10 min and 20 µL of the resulting supernatants were used for HPLC analysis as previously described by Gilmore and Yamamoto (1991) using the previously mentioned HPLC system. Solvent A (acetonitrile, methanol, Tris-HCl buffer 0.1M pH 8.0, 7.2, 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 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.

#### Individual phenolic compounds

The individual phenolic compounds were extracted in 80% methanol as described by Szauer-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).

#### Statistical analysis

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

#### RESULTS

#### **Growth parameters**

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 has shifted off, to some extent, the inhibitory effect of salinity on various growth parameters of tomato fruit (Table 1).

#### ASA and Na contents

Salt stress in SNP-treated and untreated plants significantly increased ASA content of tomato fruit by 1.97 and 7.09 fold, respectively compared to their controls (Table 2). It resulted also in an increase in the Na content in both SNP-treated and untreated plants. However, spraying salinized tomato with SNP decreased the Na<sup>+</sup> accumulation in fruit tissues by 63.5 % compared to salinized plants (Table 2).

#### Lycopene and -carotene

Under the prevailing experimental conditions, salinity decreased -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 (110 %) and that was associated with a Significant decrease in -carotene (68.3 %) while it has almost no or little effect under salt stress.

**Table (1):** Effect of exogenously sprayed SNP (10  $\mu$ M) on growth and 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±SD; means followed by different letters are significantly different at *P* 0.05 according to the least significant difference (LSD).

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

## Total falavonoid, Alkaloid, phenolic and anthocyanin content

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 97.9%, respectively compared to control (Table 3). Under the prevailing experimental conditions, salinity decreased the anthocyanin content while SNP treatment significantly increased it. Data in Table 3 demonstrate also that spraying the salinized tomato plants with SNP resulted in an increase in total phenolic content while the total alkaloids remains almost constant compared to unsprayed ones.

#### Individual phenolic compounds

Under the prevailing experimental conditions, salinity increased some individual phenolic acids such as 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 may suggest that salinity enhances the biosynthesis of these acids at the expense of their precursors (cinnamic and coumaric).

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

	Na content mmol g <sup>-1</sup> FW	ASA μg g <sup>-1</sup> FW	Lycopene mg g <sup>-1</sup> FW	-carotene μg g <sup>-1</sup> FW
Hoagland	$1.80{\pm}0.109^{b}$	11.19±1.095 <sup>b</sup>	$16.91 \pm 1.42^{b}$	1.89±0.109 <sup>a</sup>
SNP only	$1.73{\pm}0.098^{b}$	23.72±3.07 <sup>c</sup>	35.57±3.07 <sup>a</sup>	$0.57{\pm}0.032^{b}$
Salt only Salt + SNP	$\begin{array}{c} 9.87{\pm}1.06^{a} \\ 3.60{\pm}0.36^{c} \end{array}$	$\begin{array}{c} 79.41{\pm}6.88^{a} \\ 46.8{\pm}5.03^{d} \end{array}$	5.99±0.69° 6.89±0.711°	0.117±0.01 <sup>c</sup> 0.197±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±SD; means followed by different letters are significantly different at *P* 0.05 according to the least significant difference (LSD).

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

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

	Phenolic compounds								
Treatment	hent $\mu g g^{-1} FW$								
	Gallic	Protocatechuic	Vanillic	Chlorogenic	Esculetin	Ferulic	Sinapic	Coumaric	Cinnamic
Hoaglands	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

For example, chlorogenic acid increased by about 55% in salt stressed tomato fruit while cinnamic acid decreased by about 23.5%. SNP had an inductive effect on some phenolic acids such as ferulic, chlorogenic and Protocatechuic

#### DISCUSSION

#### **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 (Mitchel *et al.*, 1991; Rahman *et al.*, 2006; Saeed and Ahmad, 2009) and strawberry (Saied *et al.*, 2005; Khayyat *et al.*, 2007) grown under saline soil. In contrast, several authors reported that fruit dry weight was significantly increased under saline condition in a number of horticultural crop species including tomato (Krauss *et al.*, 2006; Gautier *et al.*, 2010), cucumber (Chartzoulakis, 1992) and sweet peppers (Janse, 1989).

#### ASA and Na content

Under the prevailing experimental conditions, the increased concentration of ASA in tomato fruits grown under saline condition 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 fruit grown under salinity. Navarro et al., (2006) reported also that salinity decreased ASA content of pepper fruit and this effect was dependent on the maturity stage. However, the contradictory results reported on the impact of salinity on ascorbate content in tomato fruit might be related to, genetic differences in the sensitivity to salinity stress, differences in the intensity of salinity applied to the plant, interactions with other factors like ripening stage (Dumas et al., 2003) and to the possibility for a plant to limit salt accumulation within its tissues (Rajasekaram et al., 2000) triggering differences in the intensity of salinity stress perceived by the plant.

Furthermore, it is well known that ascorbate 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). Furthermore, salt stress significantly increased the sodium content of tomato fruit (Table 2) which may result in an enhancement of oxidative parameters (Zushi *et al.*, 2009; Gautier *et al.*, 2010). Consequently, in the present study increased ASA content under salt stress might be linked to the key role of ascorbate as a non enzymatic system and a strong antioxidant in response to the salinity induced oxidative damage.

Spraying salinized tomato with SNP decreased the  $Na^+$  accumulation in fruit tissues and this indicates that SNP may protect plasma membrane integrity against the lipooxygenative processes.

#### Lycopene and -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 -carotene are widely known as powerful natural antioxidants that act as the most efficient singlet oxygen quencher in vitro among common carotenoids (Di Mascio et al., 1989). In the present study, salinity decreased -carotene and lycopene contents of tomato fruits (Table 2). In agreement with these data, Dorais et al., (2000) showed that -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 no effect on -caroten.

In contrast, Petersen *et al.*, (1998) and Krauss *et al.*, (2006) have reported that moderate salinity enhances lycopene and -carotene in fresh tomato fruit, although this was not confirmed by the results of Fernández-Garcia *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 (Hornero Mendez et al., 2000) and it is well known that salinity suppresses photosynthesis (Chartzoulakis and Klapaki, 2000; Bethke and Drew, 1992). Thus, under the prevailing experimental conditions the decrease in lycopene and -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 -carotene (Giuliano et al., 1993; 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 -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 -carotene while it has almost no or little effect under salt stress. Therefore, SNP treatment alone may block the enzymatic activities of carotene biosynthesis (eg. -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.

# Total flavonoid, Alkaloid, phenolic and anthocyanin content

The results of the present study on phenol contents are in conformity with the findings in pepper (Navarro *et al.*, 2006) and tomato fruit (Krauss *et al.*, 2006) while it contrasts with those of Maggio *et al.*, (2007) working with 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 which are not usually synthesized in tomato fruits (Torres *et al.*, 2005; 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 level of anthocyanin and flavonol glycosides in pea leaves which was 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 the stress conditions, i.e., against oxidative burden since salt stress was found to be accompanied by an increased production of reactive oxygen species (Rezazadeh *et al.*, 2102).

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

#### 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 on several other plants such as artichoke leaves and tomato fruit (Sgherri *et al.*, 2007; Rezazadeh *et al.*, 2012) grown under saline conditions as well as water stressed *Ctenanthe setosa* leaves (Ayaz, *et al.*, 1999). Furthermore, several types of wounding of apple fruits and leaves have been found to induce accumulation of chlorogenic acid and flavanols via activating PAL (Mayr *et al.*, 1995; Michalek *et al.*, 1999).

The results of the present study may also suggest that salinity enhances the biosynthesis of these acids as salt stress induced components that could play an important role to diminish the oxidative processes. These results support the theory that polyphenols as secondary metabolites protect the plant tissues against oxidative stress generated by salinity and contribute in salinity tolerance. It has been known that phenolic compounds of fruit may contribute to antioxidant intake, presumed to have a health protective action (Kroon *et al.*, 1999). For example, recent research indicated that benzoic and cinnamic acid derivatives have been recognized 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 of some phenolic acids under salt condition may confirm the hypothesis that NO can act as an inducer for secondary metabolites biosynthesis (total phenolics and anthocyanin) which act as oxygen scavengers to reduce the oxidative stress.

#### References

- ADEDEJI, O., K.A. TAIWO, C.T. AKANBI, AND R. AJANI. 2006. Physiochemical properties of four tomato cultivars growing in Nigeria. Journal of Food Processing and Preservation **30**: 79-86.
- AYAZ, F.A., A. KADIOGLU, AND R.TURGUT. 1999. Water stress effects on the content of low molecular weight carbohydrates and phenolic acids in *Ctenanthe setosa* (Rosc.) Eichler. Can. Journal of Plant Science **80**: 373-378.
- AZOOZ, M.M., M.A. SHADDAD, AND A.A. ABDEL-LATEF. 2004. Leaf growth and  $K^+/Na^+$  ratio as an indication of the salt tolerance of three sorghum cultivars grown under salinity stress and IAA treatment. Acta Agronomy Hungarica **52**:287–296.
- BABU, M.A., D. SINGH, AND K.M. GOTHANDAM. 2011. Effect of salt stress on expression of carotenoid pathway genes in tomato. Journal of Stress Physiology and Biochemistry **7**:87-94.
- BALIBREA, M.E., J. DELL'AMICO, M.C. BOLARIN, AND F. PEREZ-ALFOCEA. 2000. Carbon partitioning and sucrose metabolism in tomato plants growing under salinity. Physiolgia Plantarum **110**: 503 511.
- BETHKE, P.C., AND M.C. DREW. 1992. Stomatal and non stomatal components to inhibition of photosynthesis in leaves of Capsicum annuum during progressive exposure to NaCl salinity. Plant Physiology **99**: 219–226.
- BOLARIN, M.C., M.T. ESTAN, M. CARO, R. ROMERO-ARANDA, AND J. CUARTERO. 2001. Relationship between tomato fruit growth and fruit osmotic potential under salinity. Plant Science **160**: 1153 – 1159.
- BUGIANESI, R., M. SALUCCI, C. LEONARDI, R. FERRACANE, G. CATASTA, E. AZZINI, AND G. MADANI. 2004. Effect of domestic cooking on human bioavailability of naringenin, chlorogenic acid, lycopene and carotene in cherry tomatoes. European Journal of Nutrition **43**: 360-366.
- BUSTAN, A., S. COHEN, Y. DE-MALACH, P. ZIMMERMANN, R. GOLAN, M. SAGI, AND D. PASTERNAK. 2005. Effect of timing and duration of brackish irrigation water on fruit yield and quality of late summer melons. Agriculture Water Management **74**: 123–134.
- CHAPMAN, H.D., AND P.F. PRATT. 1982. Methods of analysis for soil, plants and water. Chapman publisher, Riverside, California.
- CHARTZOULAKIS, AND G. KLAPAKI.2000. Respon-

se of two greenhouse pepper hybrids to NaCl salinity during different growth stages. Scientia Horticulturae **86**:247–260.

- CHARTZOULAKIS, K.S. 1992. Effects of NaCl salinity on, germination, growth and yield of greenhouse cucumber. Journal of Horticultural Science **67**: 115-119.
- DE PASCALE, S., A. MAGGIO, V. FOGLIANO, P. ABROSINO, AND A. RITIENI. 2001. Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. Journal of Horticultural Science and Biotechnology **76**(4): 447–453.
- DI MASCIO, P., S. KAISER, AND H. SIES. 1989. Lycopene as the most efficient biological carotenoid single oxygen quencher. Archives of Biochemistry and Biophysics **274**:532–538.
- DIXON, R.A., AND N.L. PALVA. 1995. Stress induced phenylpropanoid metabolism. Plant Cell **7**: 1085-1097.
- DORAIS, M., D.L. EHRET, AND A.P. PAPADOPOULOS. 2008. Tomato (*Solanum lycopersicum*) health components, from the seed to the consumer. Phytochemistry Review **7**:231–250.
- DORAIS, M., G. TURCOTTE, A.P. PAPADOPOULOS, X. HAO, AND A. GOSSELIN. 2000. Control of tomato fruit quality and flavour by EC and water management. Agriculture and Agri-Food Canada report: pp 18–21.
- DUMAS, Y., M. DADOMO, G. DI LUCCA, AND P. GROLIER. 2003. Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. Journal of Science Food and Agriculture **83**:369–382.
- ERHARDT, J.G., C. MEISNER, J.C. BODE, AND C. BODE. 2003. Lycopene, -carotene, and colorectal adenomas. American Journal of Clinical Nutrition **78**:1219–1224.
- FANASCA, S., A. MARTINO, E. HEUVELINK, AND C. STANGELLINI. 2007. Effect of electrical conductivity, fruit pruning, and truss position on quality in green house tomato fruit. Journal of Horticultural Science and Biotechnology **82**: 488-494.
- FERNÁNDEZ –GARCIA, N., V. MARTINEZ, A. CERDA, AND M. CARVAJAL. 2004. Fruit quality of grafted tomato plants grown under saline conditions. Journal of Horticultural Science and Biotechnology **79**:995– 1001.
- FISH, W.W., P. PERKINS-VEAZIE, AND J.K. COLLINS. 2002. A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. Journal of Food Composition and Analysis **15**: 309–317.
- GAHLER, S., K. OTTO, AND V. BOHM. 2003. Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products. Journal of Agriculture and Food *Chemistry* **51**:7962-7968.
- GANJEWALA, D., S. BOBA, AND A.S. RAGHAVENDRA. 2008. Sodium nitroprusside affects the level of anthocyanin and flavonol glycosides in pea (*Pisum sativum* L. cv. *Arkel*) leaves. Acta Biologica Szegediensis **52**(2): 301-305.

- GAO, Z., M. SAGI, AND S.H. LIPS. 1998. Carbohydrate metabolism in leaves and assimilate partitioning in fruits of tomato (*Lycopersicon esculentum* L.) as affected by salinity. Plant Science **135**: 149 159.
- GARCIA-SALAS, P., A. MORALES-SOTO, A. SEGURA-CARRETERO, AND A. FERNÁNDEZ-GUTIÉRREZ. 2010. Phenolic-compound-extraction systems for fruit and vegetable samples. Molecules **15**: 8813-8826.
- GAUTIER, H., F. LOPEZ-LAURI, C. MASSOT, R. MURSHED, I. MARTY, D. GRASSELLY, C. KELLER, H. SALLANON, AND M. GENARD. 2010. Impact of ripening and salinity on tomato fruit ascorbate content and enzymatic activities related to ascorbate recycling. Functional Plant Science and Biotechnology **4**: 66-75.
- GILMORE, A.M., AND H.Y. YAMAMOTO. 1991. Linear models relating xanthophylls and lumen acidity to non-photochemical fluorescence quenching. Evidence that antheraxanthin explains zeaxanthin-independent quenching. Photosynthesis Research **35**: 67–78.
- GIULIANO, G., G.E. BARTLEY, AND P.A. SCOLNIK.1993. Regulation of carotenoids biosynthesis during tomato development. Plant Cell **5**: 379–387.
- GOSSETT, D.R., E.P. MILLHOLLON, AND M.C. LUCAS. 1994. Antioxidant response to NaCI stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Science **34**: 706-714.
- HASEGAWA, P.M., R.A. BRESSAN, J.K. ZHU, AND H.J. BOHNERT. 2000. Plant cellular and molecular response to high salinity. Annual Review of Plant Physiology and Plant Molecular Biology **51**:463-499.
- HORNERO-MENDEZ, D., R. GOMEZ-LADRON, AND M.I. MINGUEZ-MOSQUERA. 2000. Carotenoid biosynthesis changes in five red pepper (*Capsicum annuum* L.) cultivars during ripening. Cultivar selection for breeding. Journal of Agriculture and Food Chemistry **48**:3857–3864.
- JALEEL, C.A., G.M.A. LAKSHMANAN, M. GOMATHINAYAGAM, AND R. PANNEERSELVAM. 2008. Triadimefon induced salt stress tolerance in *Withania somnifera* and its relationship to antioxidant defense system. South African Journal of Botany **74**: 126–132.
- JANSE, J. 1989. Effects of humidity, temperature and concentration of the nutrient solution on firmness, shelflife and flavor of sweet pepper fruits (*Capsicum annum* L.). Acta Horticulturae **244**: 123-132.
- JUNG, S. 2004. Variation in antioxidant metabolism of young and mature leaves of *Arabidopsis thaliana* subjected to drought. Plant Science **166**: 459-466.
- KAO, W.Y., T.T. TSAI, AND C.N. SHIH. 2003. Photosynthetic gas exchange and chlorophyll *a* fluorescence of three wild soybean species in response to NaCl treatments. Photosynthetica **41**: 415-419.
- KEUTGEN, A.J., AND P. PAWELZIK. 2008. Quality and nutritional value of strawberry fruit under long term salt stress. Food Chemistry **107**:1413–1420.
- KHAN, M.N., M.H. SIDDIQUI, F. MOHAMMAD, M. NAEEM, AND M.M.A. KHAN. 2010. Calcium chloride and gibberellic acid protect Linseed (*Linum usitatissimum* L.) from NaCl stress by inducing

antioxidative defense system and osmoprotectant accumulation. Acta Physiolgia Plantarum **32**:121–132.

- KHAYYAT, M., E. TAFAZOLI, S. ESHGHI, M. RAHEMI, AND S. RAJAEE. 2007. Salinity, supplementary calcium and potassium effects on fruit yield and quality of strawberry (*Fragaria ananassa* Duch.). American-Eurasian Journal of Agricultural and Environmental Science **2** (5): 539-544.
- KRAUSS, S., W. SCHNITZLER, J. GRASSMANN, AND M. WOLTIKE. 2006. The influence of different electrical conductivity values in a simplified recirculating soilless system on inner and outer fruit quality characteristics of tomato. Journal of Agriculture and Food Chemistry **54**:441-448.
- KROON, P.A., AND G. WILLIAMSON. 1999. Hydroxycinnamates in plants and food, current and future perspectives, review. Journal of the Science of Food and Agriculture **79**: 355-361.
- LUXIMON-RAMMA, A., T. BAHORUM, M.A. SOOBRATEE, AND O.I. ARUOMA. 2002. Antioxidant activities of flavonoid componoids in extracts of *Cassia fistula*. Journal of Agriculture and Food Chemistry **50**: 5042-5047.
- MAGGIO, A., S. DE PASCALE, M. FAGNANO, AND G. BARBIERI. 2007. Can salt stress-induced physiological responses protect tomato crops from ozone damages in Mediterranean environments? European Journal of Agronomy **26**:454–461.
- MANCINELLI, A.L., C.H. YANG, I. RABINO, AND K. KUZMANOFF. 1976. Photo control of anthocyanin synthesis. Plant Physiology **58**: 214-219.
- MARTÍNEZ-VALVERDE, I., M.J. PERIAGO, G. PROVAN, AND A. CHESSON. 2002. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). Journal of the Science of Food and Agriculture **82**:323-330.
- MAYR, U., S. FÜNFGELDER, D. TREUTTER, AND W. FEUCHT. 1995. Induction of phenol accumulation by pesticides under the control of environmental factors, in: Manka, M. (eds.), Environmental biotic factors in integrated plant disease control, Poznan, pp. 399–402.
- MCDONALD, S., P.D. PRENZLER, M. ANTOLOVICH, AND K. ROBARDS. 2001. Phenolic content and antioxidant activity of olive extracts. Food Chemistry **73**: 73-84.
- Mes, P.J., P. Boches, AND J.R. Myers. 2008. Characterization of tomatoes expressing anthocyanin in the fruit. Journal of the American Society for Horticultural Science **133**: 262–269.
- MICHALEK, S., C. KLEBEL, D. TREUTTER, AND W. FEUCHT. 1999. Stimulation of flavanol biosynthesis in apple (*Malus domestica*) by abiotic elicitors. Acta Horticulturae **484**:535-539.
- MITCHELL, J.P., C. SHENNAN, AND S.R. GRATTON. 1991. Tomato fruit yields and quality under water deficit and salinity. Journal of the American Society for Horticultural Science **116**:215-221.

- MUNNS, R. 2002. Comparative physiology of salt and water stress. Plant Cell Environment **25**: 239-250.
- MURANAKA, S., K. SHIMIZU, AND M. KATO. 2002. Ionic and osmotic effects of salinity on single-leaf photosynthesis in two wheat cultivars with different drought tolerance. Photosynthetica **40**: 201-207.
- MURPHY, K.S.T., AND M.J. DURAKO. 2003. Physiological effects of short term salinity changes on *Ruppia maritima*. Aquatic Botany **75**:293-309.
- NASIBI, F., AND K.M. KALANTARI. 2009. Influence of nitric oxide in protection of tomato seedling against oxidative stress induced by osmotic stress. Acta Physiologia Plantarum **31**: 1037–1044.
- NATELLA, F., M. NARDINI, M. DI FELICE, AND C. SCACCINI. 1999. Benzoic and cinnamic acid derivatives as antioxidants, structure-activity relation. Journal of Agriculture and Food Chemistry **47**: 1453-1459.
- NAVARRO, J.M., C. GARRIDO, M. CARVAJAL, AND V. MARTÍNEZ. 2002. Yield and fruit quality of pepper plants under sulphate and chloride salinity. Journal of Horticultural Science and Biotechnology **77**:52-57.
- NAVARRO, J.M., P. FLORES, C. GARRIDO, AND V. MARTÍNEZ. 2006. Changes in antioxidant compounds content in different ripening stages of pepper fruits affected by salinity. Food Chemistry **96**: 66-73.
- PARIDA, A., A.B. DAS, Y. SANADA, AND P. MOHANTY. 2004. Effects of salinity on biochemical components of the mangrove *Aegiceras corniculatum*. Aquatic Botany 80: 77-87.
- PETERSEN, K.K., J. WILLUMSEN, AND K. KAACK. 1998. Composition and taste of tomatoes as affected by increased salinity and different salinity sources. Journal of Horticultural Science and Biotechnology **73**:205–215.
- RAHMAN, M.J., M.S. UDDIN, S.A. BAGUM, A.T.M.A.I. MONDOL, AND M.M. ZAMAN. 2006. Effect of mulches on the growth and yield of tomato in the costal area of Bangladesh under rainfed condition. International Journal Sustainable Crop Production **1**:6-10.
- RAJASEKARAM, L.R., D. ASPINALL, AND L.G. PELG. 2000. Physiological mechanism of tolerance of *Lycopersicon* spp. exposed to salt stress. Canadian Journal of plant Science **80**:151-159.
- RASHIDI, M., AND K. SEYFI. 2007. Classification of fruit shape in cantaloupe using the analysis of geometrical attributes. World Journal of Agriculture Sciences **3**(6): 735-740.
- REZAZADEH, A., A. GHASEMNEZHAD, M. BARANI, AND T TELMADARREHEI. 2012. Effect of salinity on phenolic composition and antioxidant activity of artichoke (*Cynara scolymus* L.) leaves. Research Journal of Medicinal Plant **6**: 245-252.
- RIGGI, E., C. PATANE, AND G. RUBERTO. 2008. Content of carotenoids at different ripening stages in processing tomato in relation to soil water availability. Australian Journal of Agricultural Research **59**:348– 353.

- ROMERO-PUERTAS, M.C., M. PERAZZOLLI, E.D. ZAGO, AND M. DELLEDONNE. 2004. Nitric oxide signaling functions in plant–pathogen interactions. Cell Microbiology **6**:795–803.
- SAEED, R., AND R. AHMAD. 2009. Vegetative growth of tomato as affected by the application of organic mulch and gypsum under saline rhizosphere. Pakistan Journal of Botany **41**(6): 3093-3105.
- SAIED, A.S., A.J. KEUTGEN, AND G. NOGA. 2005. The influence of NaCl salinity on growth, yield and fruit quality of strawberry cvs. "*Elsanta*" and "*Korona*". Scientia Horticulturae **103**:289-303.
- SAITO, T., N. FUKUDA, AND S. NISHIMURA. 2006. Effects of salinity treatment duration and planting density on size and sugar content of hydroponically grown tomato fruits. Journal of the Japanese Society for Horticultural Science **75**:392 – 398.
- SAITO, T., C. MATSUKURA, Y. BAN, K. SHOJI, M. SUGIYAMA, N. FUKUDA, AND S. NISHIMURA. 2008. Salinity stress affects assimilate metabolism at the gene expression level during fruit development and improves fruit quality in tomato (*Solanum lycopersicum L.*). Journal of the Japanese Society for Horticultural Science **77:** 61 68.
- SAYED, O.H. 2003. Chlorophyll fluorescence as a tool in cereal crop research. Photosynthetica **41**:321-330.
- SGHERRI, C., F. NAVARI-IZZO, A. PARDOSSI, G.P. SORESSI, AND R. IZZO. 2007. The influence of diluted seawater and ripening stage on the content of antioxidants in fruits of different tomato genotypes. Journal of Agriculture Food Chemistry **55**:2452-2458.
- SHI, J., M.L.E. MAGUER, AND M. BRYAN. 2002. Functional foods; biochemical and processing aspects. CRC Press
- SIDDIQUI, M.H., F. MOHAMMAD, AND M.N. KHAN. 2009. Morphological and physio-biochemical characterization of *Brassica juncea* L.Czern. and Coss. genotypes under salt stress. Journal of Plant Interaction **4**: 67–80.
- SINGH, D.K., B. SRIVASTVA, AND A. SAHU. 2004. Spectrophotometric determination of Rauwolfia alkaloids, estimation of reserpine in pharmaceuticals. Analytical Science **20**: 571-573.
- STEEL, R.C., AND J.H. TORRIE. 1980. Principles and procedures of statistics, 2<sup>nd</sup> ed. Mc Graw Hill, New York.

- SZAUER-HAJDRYCH, M., W. BYLKA, I. MATLAWSKA, M. WOJCIAK-KOSIOR, G. MATYSIK, AND J. JODYNIS-LIEBERT. 2008. Densitometric HPTLC and HPLC analysis of phenolic acids from *Aquilegia vulgaris*. Acta Chromatographica **20** (4):685–695.
- TANG, F. Y., C. J. SHIH, L. H. CHENG, H.J. HO, AND H.J. CHEN. 2008. Lycopene inhibits growth of human colon cancer cells via suppression of the Akt signaling pathway. Molecular Nutrition and Food Research **52**:646–654.
- TAYLOR, N.L., D.A. DAY, AND A.H. MILLAR. 2004. Targets of stress-induced oxidative damage in plant mitochondria and their impact on cell carbon/nitrogen metabolism. Journal of Experimental Botany **55**:1–10.
- TIWARI, J.K., A.D. MUNSHI, R. KUMAR, R.N. PANDEY, A. ARORA, J.S. BHAT, AND A.K. SUREJA. 2010. Effect of salt stress on cucumber, Na<sup>+</sup>–K<sup>+</sup> ratio, osmolyte concentration, phenols and chlorophyll content. Acta Physiologia Plantarum **32**:103–114.
- TORRES, C.A., N.M. DAVIES, J.A. YANEZ, AND P.K. ANDREWS. 2005. Disposition of selected flavonoids in fruit tissues of various tomato (*Lycopersicon esculentum* Mill.) genotypes. Journal of Agriculture and Food Chemistry **53**: 9536–9543.
- WENDEHENNE, D., J. DURNER, AND D.F. KLESSIG. 2004. Nitric oxide, a new player in plant signaling and defence responses. Current Opinion Plant Biology 7:449–455.
- WU, X., W. ZHU, H. ZHANG, H. DING, AND H.J. ZHANG. 2011. Exogenous nitric oxide protects against saltinduced oxidative stress in the leaves from two genotypes of tomato (*Lycopersicom esculentum* Mill.). Acta Physiologia Plantarum **33**:1199–1209.
- YILDIRIM, E., A.G. TAYLOR, AND T.D. SPITTLER. 2006. Ameliorative effects of biological treatments on growth of squash plants under salt stress. Scientia Horticulturae **111**: 1–6.
- ZHENG, C., D. JIANG, T. DAI, Q. JING, AND W. CAO. 2010. Effects nitroprusside, a nitric oxide donor, on carbon and nitrogen metabolism and the activity of the antioxidation system in wheat seedlings under salt stress. Acta Ecologica Sinica **30**: 1174–1183.
- ZUSHI, K., N. MATSUZOE, AND M. KITANO. 2009. Developmental and tissue-specific changes in oxidative parameters and antioxidant systems in tomato fruits grown under salt stress. Scientia Horticulturae **122**:362–368.

Received September 13, 2012 Accepted October 2, 2012

Tomato fruit quality as influenced by salinity and nitric oxide

### تأثير الملوحة واك يد النتيريك على جودة ثمرة الطماطم

هالة عزت محمد اسماعيل سم النبات، كلية العلوم، جامعة الاسكندرية، ، الأسكندرية .

نوقشت في هذه الدراسة تثير الملوحة على مدى جودة الثمار في الطماطم سوبر سترين بي بستخدام محلول كلوريد الصوديوم كسيد النيتريك محلول نيتروبروسيد الصوديوم مكانية مقاومة النبات للإجهاد الملحي و تقليل ترثيره ب ميكر ومول وذلك في ظل الظروف الطبيعية في الحقل .

ثمار بيتا كاروتين والليكوبين عند ري النباتات بمحلول مغذى يحتوى على التركيز المختار من كلوريد الصوديوم وصاحب هذا النقص زيادة في محتوى الثمرة من الصوديوم والقلويدات و الفلافونيدات وكذلك سكوربيك . يضأ زيادة في بعض المركبات الفينولية من بعض احماض الفردية عند المعاملة بالملح فقد دون الرش بمحلول صوديوم نيتر وبر وسيد وهذه المركبات لها دور هام كمضادات للأ جهاد الملحي.

لى تحسين نوعية الثمار ودرجة جودتها نتيجة الرش بمحلول نيتروبروسيد الصوديوم والتغلب على الإجهاد ملحى الناشىء عن كلوريد الصد ديوم نتيج في تحسن معاملات النمو والنوعي

.