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Research Report

Mineral Composition of 'Selva' Strawberry as Affected by Time of Application of Nitric Oxide under Saline Conditions

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Abstract. The objective of present study was to evaluate the impact of time of application of nitric oxide (NO) on mineral composition of strawberry 'Selva' plants under saline conditions. Well-rooted daughter plants were planted in 3L plastic pots filled with 1:1 (v/v) ratio of peat moss and perlite and grown under the greenhouse conditions (21 ± 2/17 ± 2°C and RH = 60 ± 5% under natural sunlight). After full establishment of plants they were divided into 10 groups: control, plants sprayed with distilled water and exposed to 40 mM NaCl salinity stress, plants sprayed with 50 or 75 μM NO solutions under non-stress conditions, and plants sprayed with 50 or 75 μM NO solutions at three different application times, one week before, at the beginning, and one week after initiation of 40 mM NaCl salt stress. Results indicated that concentrations of macro-nutrients, Fe and Zn in shoots and roots were decreased due to salinity stress. The NO application, regardless of time of application and level, mitigated the deleterious effect of salinity on minerals uptake. Time aspect of NO application was important as plants received 75 μM NO solution, one week before initiation of salt stress had higher shoot N, K, and Ca concentration, productivity and leaf relative water content as compared with those received NO solution at the same concentration, one week after exposure to salt stress. Higher K/Na ratio of shoot was also observed in plants treated with 75 μM NO solution one week before start of salinity compared with salt-stressed, non-NO-treated plants. It seemed that time of NO application could change the strategy of plant against stress.

***Additional key words*:** *Fragaria ananassa*, growth, macronutrients, NaCl, sodium nitroprusside

Introduction

Plant performance may be deleteriously affected by salinity. The effect of salinity on nutrient availability, competitive uptake, transport or partitioning within plant can result in growth reduction (Grattan and Grieve, 1999; Hassanuzaman et al., 2013). Salinity modifies binding, retention and transformation of nutrients and affects their uptake thus leading to reduced plant growth (Wahome, 2001). Due to interaction between Na^+ and NH_4^+ and/or between Cl^- and NO_3^- total N uptake decreases (Rozeff, 1995). Increased level of Na^+ causes reduction in K and Ca concentrations in plant tissues (Hu and Schmidhalter, 2005). This might be due to either the antagonism of Na and K in the roots, the influence of Na on K transport into xylem or the inhibition of uptake processes (Suhayda et al., 1990). Micronutrient deficiencies are very common under salt stress (Zhu et al., 2004).

Nitric oxide (NO), an important bioactive molecule, is involved in the regulation of various physiological and biochemical processes in plants (Siddiqui et al., 2011). The exogenous sodium nitroprusside, an NO donor, significantly alleviated the oxidative damage of salinity in rice seedlings (Uchida et al., 2002), and increased the dry weight of maize and *Kosteletzkya virginica* seedlings (Guo et al., 2009). Several experiments using NO donors and inhibitors indicated plasma membrane H^+-ATPase, vacuolar H^+-ATPase and H^+-PPase mediated increase in the cytosolic K/Na ratio. Therefore, NO serves as a signal for inducing salt resistance in plants (Shi et al., 2007; Zhang et al., 2006; Zhao et al., 2004).

Strawberry cultivation and production is an ever-increasing industry. This species is highly sensitive to saline conditions (Yilmaz and Kina, 2008). Previous studies indicate that an increased level of soil salinity may lead to necrosis of strawberry plant tissues and reduction of yield and fruit quality traits (Keutgen and Keutgen, 2003; Saied et al., 2005). Sensitivity of strawberry to salt salinity can be manipulated by application

of NO, and there are many studies that indicate the beneficial impact of NO on plant growth under saline conditions. However, the potential for a temporal influence on the efficacy of NO application time, before, during, and after stress induction, has not been studied. The goal of this study was to evaluate the effect of NO application time on mineral composition in strawberry 'Selva' plants. This study will allow the assessment of the time point at which maximum of benefit of NO application occurs.

Materials and Methods

Plant Growth Conditions and Treatments

Uniformly rooted daughter plants of strawberry 'Selva' were planted in 3 L plastic pots filled with 1:1 (v/v) ratio of peat moss and perlite. After establishment of plants (after 7 weeks) when they had 4 or 5 fully expanded leaves, they were divided into 10 groups: 1, Control; 2, plants sprayed with 50 NO solution under non-stress conditions (NO50); 3, plants sprayed with 75 μM NO solution under non-stress conditions (NO75); 4, plants sprayed with 50 μM NO solution 7 days (one week) before initiation of 40 mM NaCl salinity stress (NO50→NaCl); 5, plants sprayed with 75 μM NO solution 7 days (one week) before initiation of 40 mM NaCl salinity stress (NO75→NaCl); 6, plants exposed to 40 mM NaCl salinity stress and sprayed with distilled water (NaCl); 7, plants sprayed with 50 μM NO solution simultaneously with initiation of 40 mM NaCl salinity stress (NO50-NaCl); 8, plants sprayed with 75 μM NO solution simultaneously with initiation of 40 mM NaCl salinity stress (NO75-NaCl); 9, plants exposed to 40 mM NaCl salinity and sprayed with 50 μM NO solution 7 days (one week) after (NaCl→NO50); and 10, plants exposed to 40 mM NaCl salinity and sprayed with 75 μM NO solution 7 days (one week) after (NaCl→ NO75). The experiment was lasted for two months. Sodium nitroprusside was used as NO donor. Plants were grown under the glasshouse conditions with natural light (> 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$) until full establishment of plants. The plants were fertigated by 150 mL (this volume of nutrient solution was selected according to RH, average temperature, sunlight and pots size) of 0.5X Hoagland's nutrient solution for 25 days and by 150 mL of 1X Hoagland's solution once a day afterwards. Surplus of solution (about 20%) was allowed to pass the containers to ensure salt stress in the root medium at given concentration but to avoid anoxia by water logging. Average day and night temperatures were 21 ± 2/17 ± 2°C. Relative humidity was about 60 ± 5%. On the 14th day of experimental period salt stress was initiated, by adding NaCl salt to nutrient solution. Salt stress continued till the end of study.

Root and Shoot Fresh and Dry Weight

For measuring root and shoot fresh (FW) and dry weight (DW), the plants were taken out of their pots at the end of experimental period and growth media around the shoot and root sections were washed carefully. The plants were weighed for FW and then oven dried for 48 hours at 70°C for DW and expressed as gram.

Macro and Micro-nutrients Concentration

Oven-dried samples of the shoot and root sections were used for determination of macro and micro-nutrients. Dried samples (0.5 g) were ground and ashed at 550°C in a porcelain crucible for 6 h. The white ash was mixed in 2 M hot HCl, filtered and finally made up to 50 mL with distilled water. Sodium (Na) and potassium (K) concentration of samples were determined using flame emission method using a Sherwood Scientific Ltd model 360 flame photometer. Atomic absorption spectrophotometer (AA 6200, double beam atomic absorption spectrophotometer, Shimadzu, Kyoto, Japan) was used to determine Ca, Mg and micronutrient element including Fe, Zn, Mn and Cu concentrations (Kalra, 1998).

Nitrogen (N) concentration was measured using the Kjeldahl digestion method (Kalra, 1998). Phosphorus (P) concentration was determined colorimetrically (Kalra, 1998). Chlorine (Cl) was measured by precipitation titration with silver nitrate (Mohr's method) (Kalra, 1998).

Leaf Relative Water Content (LRWC)

Leaf relative water content was measured by using ten leaf discs. The leaf discs of each treatment were weighed (FW). The leaf disks were held in distilled water until saturation for 48 h at 5°C in darkness (TW). Leaf discs were oven dried for 48 hours at 70°C (DW). Relative water content was calculated according to the following formula. This parameter was measured weekly and an average was reported:

$$LRWC\ (\%) = (FW\text{-}DW)/(TW\text{-}DW) \times 100$$

Electrolyte Leakage

Electrolyte leakage was determined by recording the electrical conductivity (EC) of leaf leachates in double distilled water at 40 and 100°C. Leaf samples (0.1 g) were cut into discs of uniform size and taken in test tubes containing 10 mL of double distilled water. The test tubes were kept at 40°C for 30 min and at 100°C in boiling water bath for 15 min and their respective electric conductivities (EC1 and EC2) were measured by conductivity-meter (Conductometer 644, Metrohm, Herisau, Switzerland).

$$Electrolyte\ leakage\ (\%) = (EC1/EC2) \times 100$$

Plant Productivity

All fruits were harvested and weighed (for two months) and total weight of harvested fruits was expressed as gram per plant.

Statistical Analysis

Treatments were conducted in a completely randomized design with four replications. Data were analyzed by SPSS 16 software and the means were compared using Duncan's multiple range test at 5% probability.

Results

Table 1 indicates the effect of 40 mM NaCl salt stress and/or time of 50 and 75 μM NO solution application on shoot and root fresh and dry weight. Salinity caused 34, 45, 43 and 46% reduction in shoot and root fresh and dry weight of plants respectively. In plants treated with NO (irrespective of NO level) one week before their exposure to salt stress, shoot FW and root FW and DW were not statistically different in comparison to the control. Plants treated with NO solution (50 or 75 μM) one week after initiation of salt stress had significantly lower shoot and root fresh and dry weight in comparison to the control but these parameters were higher compared with the salt-stressed, non-NO-treated plants.

Tables 2 and 3 indicate the effect of salt stress and/or time of 50 and 75 μM NO solution application on shoots and roots macro and micro-nutrients concentrations. Concentrations of macronutrients, Fe and Zn of non-NO-treated plants were decreased when plants were exposed to saline conditions. In plants treated with NO75→NaCl shoot N concentration was higher compared with plants treated with NO solution (irrespective of NO level) one week after initiation of salt stress. P concentration was decreased in shoots and roots of salt-stressed plants sprayed with distilled water and did not change

Table 1. The effect of 40 mM NaCl salinity stress and time of application of 50 or 75 μM NO solution sprays on 'Selva' strawberry, shoot and root fresh (FW) and dry weight (DW).

Treatments	Shoot FW (g)	Root FW (g)	Shoot DW (g)	Root DW (g)
Control	56.9 ± 1.1 ab[z]	63.2 ± 2.2 a	12.1 ± 0.3 a	12.7 ± 1.7 ab
Salinity	37.2 ± 2.2 e	34.6 ± 2.7 e	6.8 ± 1.8 d	6.7 ± 1.0 d
NO50	58.5 ± 2.0 a	63.8 ± 1.8 a	11.9 ± 0.9 ab	13.6 ± 0.5 a
NO75	58.0 ± 1.3 a	62.7 ± 1.7 a	12.5 ± 0.7 a	13.1 ± 1.0 ab
NO50→NaCl	56.6 ± 2.3 ab	63.4 ± 2.0 a	11.8 ± 0.6 ab	12.7 ± 0.8 ab
NO75→NaCl	54.8 ± 1.6 b	61.5 ± 2.1 ab	11.5 ± 0.5 b	12.2 ± 1.1 ab
NO50-NaCl	58.4 ± 1.8 a	57.1 ± 2.1 c	11.7 ± 0.1 b	12.0 ± 1.0 b
NO75-NaCl	56.5 ± 2.1 ab	59.4 ± 1.5 bc	11.3 ± 0.5 b	11.9 ± 1.4 b
NaCl→NO50	47.9 ± 1.7 d	45.4 ± 2.4 d	10.0 ± 0.7 c	9.1 ± 1.2 c
NaCl→NO75	50.6 ± 1.8 c	46.1 ± 1.9 d	10.1 ± 0.6 c	9.7 ± 1.0 c

[z]Means followed by the same letters within columns are not different at 5% probability using Duncan's test. -1, 0 and + 1 indicate the application timing (-1: one week before, 0: simultaneously and + 1 one week after initiation of salinity stress), 50 and 75 are concentrations (μM) of the NO solution. All data indicated Mean ± standard error (n = 4).

Table 2. The effect of 40 mM NaCl salinity stress and time of application of 50 or 75 μM NO solution sprays on concentrations of some macro- and micro-nutrients in 'Selva' strawberry shoot.

Treatments	N	P	K	Ca	Mg	Na	Cl	Fe	Zn	Mn	Cu
	(mg·g^{-1} DW)							(mg·kg^{-1} DW)			
Control	24.5 ± 1.0a[z]	3.3 ± 0.2a	18.8 ± 0.8b	8.5 ± 0.3a	3.9 ± 0.3a	1.7 ± 1.0c	2.5 ± 1.0d	40 ± 2.1ab	86 ± 2.1a-c	247 ± 7.1bc	50 ± 7.8a
Salinity	18.7 ± 1.2e	2.6 ± 0.1b	12.1 ± 0.9d	6.7 ± 0.2d	3.1 ± 0.2b	6.8 ± 1.4a	8.5 ± 0.8a	28 ± 2.05d	523.4f	241 ± 5.1c	51 ± 7.4a
NO50	23.8 ± 1.7a-c	3.4 ± 0.3a	20.8 ± 1.1a	8.7 ± 0.2a	3.90 ± 0.20a	1.5 ± 1.1c	2.0 ± 0.9d	41 ± 2.2a	90 ± 2.4ab	270 ± 10.1a	61 ± 8.6a
NO75	24.2 ± 1.3a-c	3.2 ± 0.2a	21.9 ± 1.3a	8.6 ± 0.2a	3.8 ± 0.1a	1.3 ± 1.4c	1.9 ± 0.7d	40 ± 2.0ab	92 ± 3.0a	266 ± 11.9ab	52 ± 6.4a
NO50→NaCl	22.4 ± 1.1a-d	3.0 ± 0.1a	15.9 ± 1.1bc	8.1 ± 0.2ab	3.6 ± 0.2ab	3.5 ± 1.3b	5.4 ± 1.0bc	36 ± 2.4bc	84 ± 3.7b-d	242 ± 8.2c	48 ± 7.9a
NO75→NaCl	23.0 ± 1.0a-c	3.1 ± 0.2a	16.2 ± 1.1b	8.2 ± 0.3ab	3.4 ± 0.2ab	3.6 ± 2.0b	5.3 ± 0.6c	37 ± 3.0a-c	82 ± 3.1cd	247 ± 8.6bc	55 ± 6.6a
NO50-NaCl	21.6 ± 1.1cd	3.2 ± 0.1a	14.3 ± 1.1bc	8.1 ± 0.3ab	3.5 ± 0.3ab	3.9 ± 1.7b	5.9 ± 0.3bc	35 ± 1.9c	77 ± 3.7de	243 ± 7.7c	56 ± 7.3a
NO75-NaCl	22.0 ± 1.2b-d	3.1 ± 0.2a	14.1 ± 0.9bc	7.9 ± 0.4bc	3.5 ± 0.4ab	4.1 ± 1.8b	6.1 ± 0.4bc	34 ± 2.8c	80 ± 4.1cd	240 ± 9.5c	60 ± 7.1a
NaCl→NO50	20.2 ± 1.3de	3.3 ± 0.2a	14.0 ± 1.0cd	7.3 ± 0.2c	3.3 ± 0.2b	4.0 ± 1.4b	6.7 ± 0.9b	33 ± 2.7c	71 ± 3.4e	244 ± 10.2bc	49 ± 8.0a
NaCl→NO75	20.4 ± 1.5de	3.2 ± 0.2a	13.8 ± 1.9cd	7.5 ± 0.3c	3.3 ± 0.2b	4.2 ± 1.5b	6.6 ± 0.9bc	32 ± 2.3c	73 ± 2.5e	247 ± 11.2bc	53 ± 7.6a

[z]Means followed by the same letters within columns are not different at 5% probability using Duncan's test. -1, 0, and + 1 indicate the application timing (-1, one week before; 0, simultaneously; and +1, one week after initiation of salinity stress), and 50 and 75 are concentrations (μM) of the NO solution. All data indicated Mean ± standard error (n = 4).

Table 3. The effect of 40 mM NaCl salinity stress and time of application of 50 or 75 µM NO solution sprays on concentrations of some macro- and micro-nutrients in 'Selva' strawberry root.

Treatments	N	P	K	Ca	Mg	Na	Cl	Fe	Zn	Mn	Cu
	(mg·g^{-1} DW)							(mg·g^{-1} DW)			
Control	23.8 ± 1.2ab[z]	1.7 ± 0.0a	22.4 ± 1.8a	2.6 ± 0.2ab	1.1 ± 0.05ab	1.8 ± 0.5de	1.6 ± 0.6c	110 ± 5.1a-c	33 ± 3.0ab	103 ± 5.4a	22 ± 3.0b
Salinity	16.9 ± 1.0d	1.3 ± 0.07b	14.1 ± 1.2c	1.8 ± 0.1e	0.7 ± 0.07e	7.1 ± 0.8a	6.8 ± 1.1a	90 ± 5.1e	19 ± 4.0d	110 ± 6.5a	25 ± 5.2b
NO50	24.4 ± 2.0a	1.8 ± 0.1a	23.9 ± 1.9a	2.7 ± 0.1a	1.1 ± 0.05ab	1.6 ± 0.9e	1.4 ± 0.2c	115 ± 6.1a	35 ± 3.2a	100 ± 6.7a	36 ± 4.0a
NO75	23.9 ± 1.1ab	1.7 ± 0.08a	22.0 ± 2.0a	2.6 ± 0.2ab	1.1 ± 0.05a	1.6 ± 0.6e	1.5 ± 0.3c	112 ± 5.2ab	37 ± 4.2a	105 ± 7.9a	34 ± 3.9a
NO50→NaCl	21.3 ± 1.5a-c	1.5 ± 0.1a	18.1 ± 1.3b	2.2 ± 0.1cd	1.0 ± .03cd	3.2 ± 0.5c-e	4.1 ± 0.6b	105 ± 6.1b-d	28 ± 2.1bc	108 ± 10.1a	23 ± 5.1b
NO75→NaCl	20.8 ± 1.4bc	1.5 ± 0.1a	18.0 ± 1.4b	2.4 ± 0.1bc	1.0 ± 0.03bc	3.3 ± 0.7cd	4.2 ± 1.0b	103 ± 5.0b-d	27 ± 3.9bc	110 ± 11.2a	27 ± 3.2b
NO50-NaCl	20.6 ± 1.4bc	1.6 ± 0.1a	17.4 ± 1.6b	2.2 ± 0.1cd	1.0 ± 0.05cd	4.2 ± 0.7bc	4.0 ± 0.8b	103 ± 5.4b-d	25 ± 4.2cd	110 ± 10.0a	22 ± 3.4b
NO75-NaCl	20.0 ± 1.1cd	1.5 ± 0.1a	18.0 ± 1.1b	2.1 ± 0.1cd	1.0 ± 0.04cd	4.1 ± 1.0bc	4.1 ± 1.1b	104 ± 6.0b-d	25 ± 4.0cd	103 ± 9.4a	25 ± 5.4b
NaCl→NO50	19.2 ± 2.0cd	1.4 ± 0.05a	17.0 ± 2.0b	2.0 ± 0.1de	1.0 ± 0.04d	4.9 ± 0.6b	4.9 ± 0.4b	98 ± 6.2de	25 ± 4.5cd	107 ± 11.0a	21 ± 4.8b
NaCl→NO75	19.7 ± 1.7cd	1.5 ± 0.07a	16.4 ± 1.0b	2.1 ± 0.1cd	1.0 ± 0.03cd	5.1 ± 1.0b	5.0 ± 0.5b	101 ± 5.7cd	23 ± 3.3cd	111 ± 9.8a	25 ± 5.0b

[z]Means followed by the same letters within columns are not different at 5% probability using Duncan's test. -1, 0, and + 1 indicate the application timing (-1, one week before; 0, simultaneously; and +1, one week after initiation of salinity stress), and 50 and 75 are concentrations (µM) of the NO solution. All data indicated Mean ± standard error (n = 4).

significantly in other treatments. The highest concentration of K in shoots was observed in plants treated with NO solution (irrespective of NO level) under non-saline conditions. Regardless of NO level, K concentration in shoots decreased, when plants were treated a week after beginning of salt stress. In plants treated with NO solution one week before (irrespective of NO level) and also NO50 simultaneously with initiation of salt-stress, shoot Ca concentration did not change significantly in comparison to control. In plants treated with NaCl →NO75 shoots and roots Ca concentration was higher than the salt-stressed, non-NO-treated plants. Root Ca concentration was significantly higher in the plants treated with NO75→ NaCl compared with NaCl→NO50. Root Mg concentration was significantly higher in all NO-treated plants than those not received NO treatments. Shoot Na concentration was significantly lower in all salt-stressed plants treated with NO solution (regardless of NO level and time of application) in comparison to salt-stressed, non-NO-treated plants. Root Na concentration in plants received NO solution one week before initiation of salt stress (irrespective of NO level) was lower in comparison to plants treated with NaCl→NO50 or NaCl →NO75. Shoots and roots Cl concentration was significantly lower in all NO-treated plants compared with untreated plants under saline conditions. Concentration of Fe in shoots was not statistically different in plants treated with NO50→ NaCl and NO75→NaCl compared with control. Shoot Fe concentration was decreased, in plants treated with NO solution (irrespective of NO level) simultaneously or one week after exposure of plants to saline conditions. Concentration of Fe in roots showed no significant difference in the plants treated with 50 and 75 µM NO solution one week before or simultaneously with initiation of salt stress and plants treated with NaCl→NO75. Shoot Zn concentration was not statistically

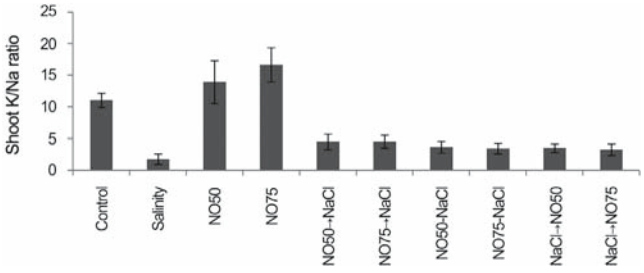

Fig. 1. K/Na ratio in 'Selva' strawberry shoots under influence of 40 mM NaCl salinity stress and/or different time of application of 50 and 75 µM NO solution. Vertical bars indicate standard errors (n=4).

different in plants treated with NO50→NaCl, NO75→NaCl and NO75-NaCl in comparison to control. Root Zn concentration of plants treated with NO (irrespective of NO level) simultaneously or one week after initiation of salinity and salt-stressed, non-NO-treated plants expressed no significant differences. In plants grown under non-saline conditions and treated with NO50 shoot Mn concentration increased significantly. Root Cu concentration increased in plants treated with NO (irrespective of NO level) under non-saline conditions in comparison to control.

Figures 1 and 2 present the effect of different time of application of NO solution (50 or 75 µM) and/or 40 mM NaCl salt stress on shoot and root K/Na ratio. The K/Na ratio was decreased in both shoots and roots of plants under saline-conditions. The highest ratio of K/Na in shoots (16.62) was observed in the plants received NO75 under non-saline conditions. Root K/Na ratio of plants treated with NO50-NaCl, NO75-NaCl, NaCl→NO50 and NaCl→NO75 and shoot K/Na ratio in the plants treated with NO75-NaCl and NaCl→ NO75 expressed no significant differences compared with

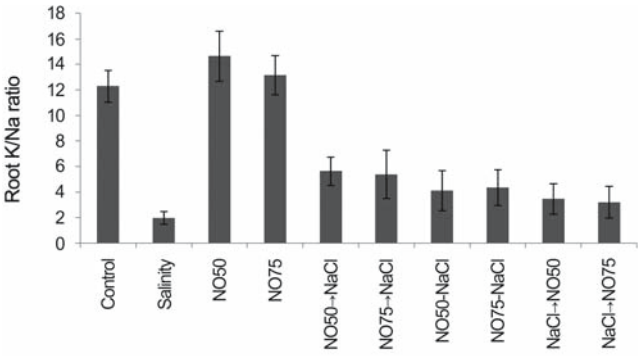

Fig. 2. K/Na ratio in 'Selva' strawberry roots under influence of 40 mM NaCl salinity stress and/or different time of application of 50 or 75 μM NO solution. Vertical bars indicate standard errors (n = 4).

the salt-stressed, untreated plants.

Table 4 shows the effect of 40 mM NaCl salinity stress and/or time of application of 50 or 75 μM NO solution on LRWC, EL and total productivity of plants. The LRWC was decreased due to salinity. In the plants treated with 50 and 75 μM NO solution one week before or simultaneously with initiation of salt stress LRWC was higher in comparison to those treated with NO (irrespective of NO level) solution one week after exposure of plants to saline condition. Leaf EL increased significantly in salt stressed, non-NO-treated plants. In the plants treated with 50 and 75 μM NO solution one week before or simultaneously with initiation of salt stress leaf EL was lower compared with salt stressed plants treated with distilled water. Productivity decreased significantly in salt-stressed, untreated plants. Highest productivity of plants was obtained from control and those treated with NO75 and NO50 under non-saline conditions.

Discussion

A vast range of studies have reported reduction of shoot and/or root fresh and dry weight after exposure of plants to saline conditions due to low osmotic potential of soil solution (water stress), nutritional imbalance, specific ion effect (salt stress) and a combination of these factors (Hassanuzaman et al., 2013). Beneficial impact of exogenous NO application on shoot and root fresh and dry weight was indicated in the present study. Exogenously-applied SNP (sodium nitroprusside), significantly enhanced *Suaeda salsa* seedlings' growth (Song et al., 2009), and increased the dry weight of maize and *Kosteletzkya virginica* seedlings under salt stress (Guo et al., 2009; Zhang et al., 2006). The NO plays an important role in mediating root elongation and development (Correa-Aragunde et al., 2004). The involvement of NO in IAA-induced adventitious root development has been reported (Pagnussat et al., 2002).

Macro and micro nutrients absorption was adversely affected by salinity which was in accordance with previous works (Hu and Schmidhalter, 1997; Hu and Schmidhalter, 2005; Rozeff, 1995) and application of NO mitigated this detrimental influence of salinity on nutrients absorption. In the calluses of reed (*Phragmites communis* Trin.), NO application increased the expression of the plasma membrane H^+-ATPase, leading to a higher K/Na ratio in the cytosol (Zhao et al., 2004). Exogenous application of NO, in maize, increased the activity of tonoplast H^+-ATPase and Na^+/H^+ antiport facilitating Na^+ compartmentation (Zhang et al., 2006). In calluses from poplar (*Populus euphratica* L.), NO increased salt tolerance by improving plasma membrane H^+-ATPase activity that increased the K/Na ratio (Zhang et al., 2007). Application of NO significantly decreased Na^+ contents and simultaneously

Table 4. The effect of 40 mM NaCl salinity stress and time of application of 50 or 75 μM NO solution sprays on productivity, leaf relative water content, and leaf electrolyte leakage in 'Selva' strawberry.

Treatments	Leaf relative water content (%)	Leaf electrolyte leakage (%)	Productivity (g/plant)
Control	82.7 ± 1.8 a[z]	20.0 ± 0.7 c	127.8 ± 4.2 a
Salinity	69.2 ± 1.4 d	26.7 ± 1.0 a	66.4 ± 2.2 d
NO50	81.0 ± 1.4 a	19.0 ± 1.0 c	126.9 ± 6.1 a
NO75	83.0 ± 1.2 a	20.2 ± 0.8 c	131.0 ± 5.4 a
NO50→NaCl	75.2 ± 1.1 b	22.2 ± 1.0 b	108.9 ± 6.6 b
NO75→NaCl	74.7 ± 1.3 b	22.2 ± 1.1 b	105.4 ± 5.8 b
NO50-NaCl	72.5 ± 1.7 bc	22.5 ± 0.9 b	103.2 ± 5.9 bc
NO75-NaCl	73.5 ± 1.1 b	23.0 ± 1.1 b	100.4 ± 6.7 bc
NaCl→NO50	69.7 ± 1.6 d	25.0 ± 0.8 a	92.5 ± 5.4 c
NaCl→NO75	71.0 ± 1.2 cd	25.7 ± 1.0 a	93.7 ± 5.0 c

[z]Means followed by the same letters within columns are not different at 5% probability using Duncan's test. -1, 0, and + 1 indicate the application timing (-1, one week before; 0, simultaneously; and +1, one week after initiation of salinity stress), and 50 and 75 are concentrations (μM) of the NO solution. All data indicated Mean ± standard error (n = 4).

increased K^+ contents in the shoots of seashore mallow, under saline conditions. Similarly, NO increased K^+ accumulation in roots, leaves and sheaths, and simultaneously decreased Na^+ accumulation in maize (Zhang et al., 2004). Fe can accumulate in large pools in the root apoplast and can be mobilized to the shoots as the plants become Fe-deficient. This translocation of Fe might be important in resistance to Fe deficiency-chlorosis (Longnecker and Welch, 1990). Graziano et al. (2002) suggested that NO might play a physiological role improving Fe translocation from roots to leaves. In addition to Fe, other micronutrients such as Mn and Zn can also be taken up by IRT1 (Connolly et al., 2002) whose expression is increased and stimulated by NO (Zhu et al., 2012).

Increase in leaf EL and decrease in LRWC under saline conditions in our study were in accordance with previous studies on strawberry (Karlidag et al., 2009; Kaya et al., 2002). The increased level of salts in root media will produce lower water potential which can results in decreased LRWC (Romero-Aranda et al., 2001). Salinity induces mineral imbalance, this leads to lower Ca^{2+} uptake which can cause loss of cellular membrane integrity and increase in leaf EL (White and Broadley, 2003). Application of NO mitigated these adverse influences of salinity on plants in our study in agreement with previous works (Bai et al., 2011; Hossain et al., 2010; Xiong et al., 2010).

Time aspect of NO application was important as plants pretreated with NO75→NaCl had higher shoot and root fresh and dry weight, shoot N, K, Ca concentration, productivity, LRWC and lower leaf EL in comparison to plants treated with NaCl→NO75. Also shoot Fe and Zn concentrations were not significantly different in plants treated with NO50 →NaCl or NO75→NaCl compared with the control. Lower Na uptake, improved K absorption and better compartmentation of absorbed Na are plant strategies to tolerate saline conditions (Hassanuzaman et al., 2013). These strategies are regulated and modified by NO (Siddiqui et al., 2011). Under saline conditions, along with impairment of K uptake, higher K levels in tissues can mask the adverse effects of salinity on growth (Grattan and Grieve, 1999). The Presence of a higher concentration of K and Ca in shoots of plants pretreated with NO can eventually mitigate some of injuries induced by salinity. Khayat et al. (2007) reported that supplementary Ca mitigated the reduction of strawberry plant growth observed under saline conditions. The salt inducible enzyme Na^+/H^+ antiporter removes Na^+ from cytosol (Chen et al., 2010). NO significantly increases the activity of vacuolar H^+-ATPase and H^+-PPase, the driving forces for Na^+/H^+ exchange (Zhang et al., 2007) which can cause a higher K/Na ratio in shoots under non-saline conditions.

The NO plays an important role (as a central component) in maintaining Fe bioavailability in plants. Ramirez et al. (2010) discussed how NO production constitutes a key response in plant Fe sensing and availability. The NO drives downstream responses to both Fe deficiency and Fe overload. Treatment with exogenous NO was shown to improve the fitness of maize and tomato plants grown under Fe deficiency (Graziano et al., 2002; Graziano and Lamattina, 2007). Under such conditions, the NO-treated plants displayed increased root hair development, higher chlorophyll contents and reduced interveinal chlorosis typical of Fe deficiency. Further supporting these findings, NO was rapidly produced in roots of plants exposed to Fe deficiency. Once produced, it initiates a Fe-starvation pathway promoting the expression of genes required for Fe uptake (Graziano and Lamattina, 2007). The NO can readily form complexes with transition metal ions such as Zn and Mn in aqueous solutions and nucleophylic compounds such as metalloproteins (Graziano et al., 2002). So exogenous NO can increase the plant ability to uptake micronutrients.

Rise in activity of antioxidant enzymes such as superoxide dismutase, glutathione reductase, peroxidase and ascorbic peroxidase has been reported by multiple authors after exogenous NO application (Guo et al., 2009; Sheokand et al., 2010; Wu et al., 2011) which also occurred in our study (data not shown). The NO can act as an antioxidant to protect plants from oxidative damage (Beligni et al., 2002). Moreover, NO application has been found to increase proline accumulation that scavenges ROS and stabilize the structure of the macromolecules (Ruan et al., 2002) we found similar augmentation of proline in the leaves (data not shown) after NO application. So NO gives the plants protections against damages induced by salinity, especially when applied prior or at least simultaneously with initiation of the stress; in other words NO can "vaccinate" plants against adverse abiotic stress conditions (Siddiqui et al., 2011). When this signaling molecule, reaches the plant before initiation of stress, it triggers an increase in antioxidants content and activity. Also NO-pretreatments can lead to a higher level of K, Ca and micronutrient absorption and reduced Na or Cl uptake. In other words after NO application plant becomes more salt tolerant. So when this augmentation of tolerance comes before the stress, salt-induced injuries might occur less frequent. Application of NO, after initiation of stress can be beneficial for the plant growth, but because the stress already has harmed the plants, the NO application may help to maintain the current conditions, inhibit worsening and even induce some recovery.

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