

# Role of Foliar Application of Nicotinic Acid and Tryptophan on Onion Plants Response to Salinity Stress

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## Abstract

This experiment was conducted at the National Research Centre, Dokki, Cairo, Egypt during 2006 and 2007 winter season to evaluate the effects of spraying with tryptophan (TP) and nicotinic acids (NA) on onion plants grown under varying degree of salinity stress (irrigation water with 3000 and 6000 ppm salinity) as compared to tap water (300 ppm) irrigation. Irrigation by high salinity water decreased the number of leaves, mean bulb diameter, fresh and dry weight of bulb and whole plant. Foliar application of TP and NA significantly increased the top height and fresh weight, and bulb dry weight. The above beneficial effects were greater in the plants sprayed with NA as compared to those of the plants received TP spray. At the lower salinity level (3000 ppm), TP was more effective than NA in mitigating the negative effects of salinity stress. At the high salinity stress (6000 ppm), however, the converse was evident. The electrolyte leakage (EL) was not influenced by 3000 ppm salinity irrigation water as compared to that of the plants which received tap water (300 ppm) irrigations. However, it increased significantly in the plants irrigated by 6000 ppm salinity water. Lipid peroxidation (LP) and EL were significantly lower in the plants sprayed with TP or tapwater. In addition, spraying of NA or TP increased the plant growth and biomass weight. Therefore, the above foliar sprays appear to mitigate the negative effects of increased salinity.

**Keywords:** onion (*Allium cepa* L.), irrigation, dilute seawater, salinity, nicotinic acid, tryptophan, chlorophyll, carotenoids, electrolyte leakage, lipid peroxidation

## 1. Introduction

Onion (*Allium cepa* L.) is an important vegetable crop in Egypt for domestic consumption and for export, which ranks second after potatoes in terms of export quantity and income. Growth and yield of Onion are negatively impacted by soil salinity (Doss, Bartolo, Davis, & Cardon, 2002; Abd El-Baky, Mohamed, & Hussein, 2003). Poor irrigation management, particularly using poor quality water, contributed to salinity buildup in agricultural soils both under long term and recent cultivation. Several investigations have demonstrated the role of amino acids and vitamins in mitigating the negative effects of abiotic and biotic stresses in various crops. Rhodes and Handa (1989) reported the role of amino acids in abiotic stress resistance. Amino acids play an important role in plant metabolism and are essential for protein content. Proline works as an osmoprotectant (Rhodes, Verslves, & Sharp, 1999). Glutamic acid is a precursor of two enzymes for proline synthesis in plants (Hu, Delauney, & Verma, 1992). Rhodes and Handa (1989) also reported the role of sulfur in amino acid metabolism as related to plant stress resistance mechanisms.

Ramaih, Geudira, and Paulsen (2003) confirmed that tryptophan as the major precursor of Indol acitic acid (IAA) in most organisms. Plants produce IAA from tryptophan through indole-3-pyruvic acid (Mashiguchi et al., 2011) (Won et al., 2011). IAA is also produced from tryptophan through indole-3-acetaldoxime in Arabidopsis (Satoko et al., 2009). El-Bassiouny (2005) demonstrated that tryptophan and nicotinamide increased IAA, Geberellic acid (GA3), cytokinens, and decreased abscicic acid (ABA) in wheat. Nicotinamide is a stress induced compound to provide defense mechanism against a specific stress. Wyszowska (1999) and El-Bassiouny (2005) observed an increase in some minerals  $K^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$  in wheat plant tissues by tryptophan treatment.

Pre-soaking the seeds of *Eruca sativa* in arginine and uric acid (precursors of polyamines) improved the seedlings survival of plants grown under salt stress (Yagi & Al-Abdulkareem, 2006). Seo et al. (2005) reported enhanced tolerance of rice plants to salt stress (40 mM of NaCl) following the application of 30  $\mu$ M jasmonic acid. Gadalla (2005) reported that tocopherols and ascorbic acid application to wheat plants grown in a clay loam soil with high salt content (15 dS/m) decreased the leaf senescence thus provided enhanced tolerance to salt stress. The objective of this study was to investigate the effects of application of nicotinic acid and tryptophan to mitigate the adverse effects of salinity water irrigation on onion plants growth, photosynthetic pigments and other metabolic processes.

## 2. Method

A pot experiment was conducted in a greenhouse at the National Research Centre, Dokki, Cairo, Egypt during 2006 and 2007 winter season. Metallic pots (35 cm diameter, 50 cm depth) were used. The inner surface of the pots was coated with three layers of bitumen to prevent direct contact between the soil and the metal. Two kg of gravel (2-3 cm diameter) was placed at the bottom of the pot, followed by 30 kg of air dried clay loam soil (sampled from 40 cm depth from Giza governorate lands).

Onion (*Allium cepa* L.) cultivar Behary Red seedlings were transplanted to the pots on Dec 1, 2006 and 2007. Soil in each pot received 3 g calcium super phosphate (6.8% P) and 1.5 g potassium sulfate (40.3% K) i.e. equivalent to 106, and 212 kg/ha of P, and K, respectively. Nitrogen was applied as ammonium sulfate (20.5 % N) at 6.86 g / pot, i.e. 488 kg/ha in two equal doses, 2 and 4 weeks after transplanting. All pots were irrigated using tap water during the first 21 days. Subsequently the salt water irrigation began. The treatments included:

1) Main Treatments: Irrigation using tap water or two dilute sea water, i.e. the salt concentrations were 300 (tap water), 3000, and 6000 ppm. In the salt water irrigation treatments, each irrigation with salt water was followed by two irrigations of tap water.

2) Sub-treatments: Foliar spray of either tryptophan (200 ppm) or nicotinic acid (200 ppm) as compared to only tap water spray (control). The above sprays were done on 21 and 35 days after planting.

On 60 days after planting, plant height was measured, and number of leaves per plant was recorded. The plants were harvested 90 days after planting. The bulb and top fresh weights, and bulb diameter were measured. The bulb and tops were sliced into small pieces, dried at 70 °C for 48 hours. The bulb, leaves and whole plant dry weights were also recorded.

Photosynthetic pigments: Chlorophyll a (Chl\_a), chlorophyll b (Chl\_b) and total carotenoids concentration were determined according to the method described by Wetstein (1957).

Lipid peroxidation: Determined by measuring malondialdehyde equivalents (MDA) using the thiobarbituric acid (TBA) method (Heath & Packer, 1968). Fresh biomass samples (100 mg) were homogenated in 1.5 ml of 25% TBA in 10% trichloroacetic acid (TCA). The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10000x g for 10 min the absorbance of the supernatant was read at 532 nm and correction for unspecific turbidity was done by subtracting the absorbance at 800 nm. A solution containing 0.25% TBA in 10% TCA was used as blank. The MDA content was calculated according to its extinction coefficient of 155 nmol malondialdehyde ml<sup>-1</sup>.

Electrolyte leakage: Leakiness of tissues was measured according to the method of Gilley and Fletcher (1997). Two segments from second true leaf in three replications for all treatments were immersed in 15 ml distilled water. The samples were equilibrated for 24 hr at room temperature and conductivity was measured using Model 32 Conductance Meter (Yellow Springs Instrument Co. Inc., Ohio, USA). After the initial measurement, the sample was placed in a boiling water bath for 30 min, cooled to room temperature, and a second reading was taken. The percent of leakage was calculated as the ratio of the initial conductivity to the conductivity after boiling.

Statistical analysis: Statistical significance of the response data were evaluated by analyses of variance (ANOVA) and mean separation tests (Snedecor & Cochran, 1990).

## 3. Results and Discussion

### 3.1 Irrigation Water Salinity

Irrigation by high salinity water significantly decreased the number of green leaves, mean bulb diameter, fresh and dry weights of bulb and whole plant (Table 1). Patel, Prasher, Donnelly, and Bonnell (2001) reported a significant reduction in Grade A and total tuber weight of Norland cultivar in saline soil. Heuer and Nadler (1998) reported that salinity damage to the plant was mainly due to accumulation of chloride and proline. Shaterian,

Waterer, Dong, and Tanino (2005) reported that responses to salinity stress was different in early- and late-maturing potato clones. Early-maturing clones accumulated  $\text{Na}^+$  in the leaf tissues while late-maturing clones generally excluded  $\text{Na}^+$  from the leaf tissues. Proline levels increased following the exposure of plants to salt stress. However, this response was not associated with salinity tolerance. Crop performance may be adversely affected by salinity-induced nutritional disorders. Salinity can directly affect nutrient uptake, i.e. increased  $\text{Na}^+$  in saline growing media can compete for  $\text{K}^+$  uptake. Likewise, increased  $\text{Cl}^-$  can compete for  $\text{NO}_3^-$  uptake. Salinity can also cause a combination of complex interactions that affect plant metabolism, susceptibility to injury or internal nutrient requirement (Grattain & Grieve, 1998).

Table 1. Effects of salinity on growth of onion plants

Salinity (ppm)	Top height (cm)	No. of leaves	Mean bulb Diameter (cm)	Fresh weight (g/ plant)			Dry weight (g/plant)		
				Top	Bulb	Whole plant	Top	Bulb	Whole plant
300 (Tap Water)	61.3	7.1	2.77	25.28	11.30	36.58	2.81	3.02	5.83
3000	60.6	6.9	2.64	21.33	7.01	28.34	2.44	2.45	4.89
6000	58.2	6.2	2.63	20.85	6.89	27.74	2.25	2.17	4.42
LSD $\leq$ 0.05	N.S	0.7	0.03	N.S.	1.91	8.24	N.S	0.32	0.37

LSD = Least Significant Difference;

NS = Non-significant.

The concentration of Chl\_a in the leaves of the plants, was greater with high salinity water irrigation as compared to that of plants irrigated by tap water (Table 2). All other parameters were not significantly influenced by varying levels of salinity in the irrigation water. Saha, Shatterjee, and Piswas (2010) reported a gradual decrease in the chlorophyll content with an increase in salt stress of pumpkin genotypes. Similar response was reported for melons (Kusvuran, 2010) and for canola (Nazarbeygi, Yazdi, Naseri, & Soleimani, 2011). Kaya, Higgs, and Kirnak (2001) noticed that chlorophyll concentration and water use in spinach was reduced significantly by high salinity. Our results are contrary to the above trends.

Table 2. Concentrations of photosynthetic pigments in leaves of onion plants as affected by varying irrigation water salinity

Salinity (ppm)	Chl_a	Chl_b	Carotenoids	Chl_a+Chl_b	Chl_a : Chl_b	(Chl_a+Chl_b) : Carotenoids
300 (Tap Water)	7.83	3.20	2.51	11.02	2.45	4.40
3000	8.06	2.87	2.79	10.93	2.81	3.93
6000	9.46	2.91	2.86	12.32	3.23	4.41
LSD $\leq$ 0.05	1.56	N.S	N.S	N.S	-	-

LSD = Least Significant Difference;

NS = Non-significant.

The electrolyte leakage was significantly greater in the plants exposed to high salinity water irrigation as compared to that of the plants irrigated with low salinity water or tap water (Table 3). Similar response was reported in rice (Lutts, Kinet, & Bouharmont, 1996), tomato (Kaya, Kirnak, & higgs, 2001a), and cucumber and pepper (Kaya, Kirnak, & higgs, 2001b). Excess NaCl in the growth medium induced structural changes in bean roots, as well as leakage of ions correlated with alterations of the cell membranes (Cachorro, Olmos, & Cerda, 1995). Kaya et al. (2001) demonstrated that membrane permeability was impaired in the plants grown under high salinity stress, which affects root membrane integrity. Our study shows that irrigation water salinity had no significant effects on lipid peroxidation.

Table 3. Effects of salinity on lipid peroxidation &amp; electrolyte leakage

Salinity (ppm)	Lipid peroxidation (MDA, nmol/g FW)	Electrolyte leakage (%)
300 (Tap Water)	7.80	48.45
3000	7.61	46.75
6000	10.82	61.53
LSD $\leq$ 0.05	N.S	1.50

LSD = Least Significant Difference;

NS = Non-Significant.

### 3.2 Foliar Application of Nicotinic Acid and Tryptophan

Foliar spray of nicotinic acid significantly increased the top height as compared to that of the plants that received tryptophan or only tap water (Table 4). Number of leaves and bulb diameter were not influenced by either tryptophan or nicotinic acid sprays as compared to those of the plants which received only tap water spray. The top or whole plant fresh weight as well as dry weights of top and bulb were greater for the plants with foliar application of typtophan or nicotinic acid as compared to that of the control plants (tap water spray).

Table 4. Effects of nicotinic acid &amp; tryptophan on growth of onion plants

Foliar Spray	Top height (cm)	No. of leaves	Mean bulb Diameter (cm)	Fresh weight (g/plant)			Dry weight (g/plant)		
				Top	Bulb	Whole plant	Top	Bulb	Whole plant
Tap Water	53.8	6.7	2.59	16.13	7.19	23.32	2.15	1.98	4.13
Tryptophan	59.2	6.9	2.52	24.54	9.29	33.83	2.84	2.95	5.79
Nicotinic acid	67.1	6.7	2.93	27.29	8.72	36.01	2.51	2.71	5.22
LSD $\leq$ 0.05	6.9	N.S	N.S	8.16	N.S	8.51	0.41	0.55	0.75

LSD = Least Significant Difference;

NS = Non-Significant.

Foda (1987), Mohamed, El-Din, and Foda (1989) and Deyab (1989) investigated the effects of nicotinic acid on plants, and demonstrated that 100 mg/L was the best concentration for increasing the plant height. Nicotinic acid is a necessary component for most physiological processes in all plant species, and is a component of several enzymes responsible for many biochemical reactions, including the biosynthesis of protein (Noctor, Queval, & Gakière, 2006) and/or IAA oxidase, amylase, and proteinase.

Foliar application of either nicotinic acid or tryptophan increased the concentration of Chl<sub>a</sub>, but not that of Chl<sub>b</sub> or carotenoids as compared to that of the plants received tap water spray (Table 5). This is in agreement with the similar response on rose geranium (*Pelargonium graveolens L.*) and *Philedendrom erubescens* reported by other researchers (Mahgoob & Talaat, 2005; El-Dahab & abd El-Azziz, 2006).

Table 5. Concentrations of Photosynthetic pigments in leaves of onion plants as affected by foliar application of nicotinic acid &amp; tryptophan

Foliar Treatment	Chl_a	Chl_b	Carotenoids	Chl_a+Chl_b	Chl_a : Chl_b	(Chl_a+Chl_b) : Carotenoids
	← (ppm) →					
Tap Water	6.15	2.93	2.48	9.07	2.10	3.65
Tryptophan	9.18	2.60	2.81	11.79	3.53	4.20
Nicotinic acid	9.31	3.45	2.86	12.76	2.70	4.46
LSD $\leq$ 0.05	2.32	N.S	N.S	2.87	-	-

LSD = Least Significant Difference;

NS = Non-Significant.

Lipid peroxidation was lower in the plants sprayed with nicotinic acid as compared to that of plants sprayed with tryptophan or tap water (Table 6). The electrolyte leakage was lower in the plants sprayed with tryptophan or nicotinic acid as compared to that in the plants sprayed with tap water.

Table 6. Effects of foliar application of nicotinic acid &amp; tryptophan on lipid peroxidation &amp; electrolyte leakage

Chemical treatment	Lipid peroxidation (MDA, nmol/g FW)	Electrolyte leakage (%)
Tap Water	9.27	57.79
Tryptophan	9.73	51.97
Nicotinic acid	7.16	46.44
LSD $\leq$ 0.05	1.89	6.74

LSD = Least Significant Difference.

Electrolyte leakage, a measure of membrane stability, increased in maize plants under salt stress. Exogenous application of non-enzymatic antioxidative compounds were found to be very effective in reducing electrolyte leakage in salt stressed maize plants. Sayed and Gadallah (2002) showed that root or shoot applied thiamine improved growth, which was found to be associated with thiamine-induced reduced membrane injury and increased leaf RWC. Excess lipid oxidation will eventually result in membrane damage. The extent of membrane damage can be quantified by ion leakage from the symplast (Rizhsk et al., 2002; Farouk, 2011).

### 3.3 Interactions Between Irrigation Water Salinity and Foliar Application of Nicotinic Acid or Tryptophan

The above interaction was significant only on fresh weight of bulb (Table 7). The bulb fresh weight was greater in the plants sprayed with nicotinic acid as compared to that of the control plants or those sprayed with tryptophan only under high salinity (6000 ppm) irrigation treatment. No significant effect was evident in the low salinity (3000 ppm) irrigation treatment. In tap water irrigation treatment, however, tryptophan spray significantly increased the bulb weight as compared to that of the other foliar treatments.

Table 7. Effect of irrigation water salinity &amp; foliar application of nicotinic acid or tryptophan on growth of onion plants

Salinity (ppm)	Foliar Spray	Top height (cm)	No. of leaves	Mean bulb diameter (cm)	Fresh weight (g/plant)			Dry weight (g/plant)		
					Top	Bulb	Whole plant	Top	Bulb	Whole plant
300 (Tap Water)	Tap Water	55.7	7.00	2.83	14.54	9.63	24.17	2.53	2.80	5.33
	Tryptophan	59.3	7.33	2.80	25.52	13.63	39.15	3.18	3.39	6.57
	Nicotinic acid	89.0	7.00	2.67	37.19	10.66	47.85	2.71	2.87	5.58
3000	Tap Water	56.7	7.00	2.23	18.02	6.60	24.62	2.03	1.82	3.84
	Tryptophan	57.3	6.77	2.40	23.44	8.22	31.66	2.55	3.09	5.64
	Nicotinic acid	67.7	7.00	3.30	22.56	6.22	28.78	2.41	2.45	4.86
6000	Tap Water	49.0	7.00	2.70	15.74	5.35	21.09	1.55	1.32	2.87
	Tryptophan	61.0	6.00	2.37	24.66	6.02	30.68	2.80	2.37	5.17
	Nicotinic acid	64.7	6.67	2.83	22.16	9.29	31.45	2.41	2.82	5.23
LSD $\leq$ 0.05		N.S	N.S	N.S	N.S	2.69	N.S	N.S	N.S	N.S

LSD = Least Significant Difference;

NS = Non-Significant.

Salinity stress is caused by an increased availability of Na and Cl, which contribute to reduced uptake of N, P, and K (Kaya et al., 2001a). Spraying Mong bean plants with arginine alleviated the harmful effects of salinity (Qados, 2010). Hassanein et al. (2008) and Khalil et al. (2009) also observed the positive role of arginine in alleviating the growth inhibition due to plant exposure to heat stress. The application of arginine significantly promoted the growth and increased the fresh and dry weights and certain endogenous plant growth regulators in bean (Nassar, Tarabily, & Sivasithamparam, 2003) and wheat (El-Bassiouny, 2005; El-Bassiouny et al., 2008). Ben Ahmed, Ben Rouina, Sensoy, Boukhriss, and Ben Abdullah (2010) emphasized that the proline supplements seem to improve salt tolerance of olive plants by amelioration of some antioxidative enzyme activities, photosynthetic activity, thus increased plant growth and plant water status under salinity stress.

Increase in salinity stress decreased the dry mass, chlorophyll, soluble and hydrolysable sugars (Amirjani, 2011), protein and soluble proteins (Vieira-Santos, Campos, Azevedo, & Caldeira, 1996) and enhanced contents of total free amino acids (Hussein, Gaballah, & El-Faham, 2005) on barley. The interaction between irrigation water salinity and foliar application of nicotinic acid or tryptophan was significant only on (Chl\_a + Chl\_b) for the 3000 ppm irrigation water treatment, as evident from a significantly greater (Chl\_a + Chl\_b) concentration in plants that received nicotinic acid spray as compared to that of the plants which received tryptophan or tap water spray (Table 8).

Table 8. Concentrations of photosynthetic pigments in leaves of onion plants as influenced by irrigation salinity &amp; foliar application of nicotinic acid or tryptophan

Salinity (PPM)	Foliar Spray	Chl_a	Chl_b	Carotenoids	Chl_a+Chl_b	Chl_a : Chl_b	(Chl_a+Chl_b) :
		← (ppm) →			Carotenoids		
300 (Tap Water)	Tap Water	6.88	2.75	2.20	9.63	2.50	4.39
	Tryptophan	7.77	2.82	2.38	10.59	2.76	4.45
	Nicotinic acid	8.82	4.02	2.95	12.84	2.19	4.36
3000	Tap Water	5.24	2.94	2.92	8.19	1.78	2.77
	Tryptophan	9.38	2.03	2.85	11.61	4.20	4.08
	Nicotinic acid	9.56	3.43	2.60	12.99	2.78	5.03
6000	Tap Water	6.31	3.08	2.34	9.40	2.05	4.02
	Tryptophan	10.36	2.76	3.20	13.12	3.75	4.11
	Nicotinic acid	9.56	2.89	3.06	12.45	3.31	4.07
LSD ≤ 0.05		N.S	N.S	N.S	4.78	-	-

LSD = Least Significant Difference;

NS = Non-Significant.

The lipid peroxidation or electrolyte leakage was not significantly influenced by interactions between irrigation water salinity and foliar application of nicotinic acid or tryptophan (Table 9).

Table 9. Effect of irrigation water salinity &amp; foliar application of nicotinic acid or tryptophan on lipid peroxidation &amp; electrolyte leakage

Salinity (ppm)	Foliar Spray	Lipid peroxidation (MDA, nmol/g FW)	Electrolyte leakage (%)
300 (Tap Water)	Tap Water	9.50	57.13
	Tryptophan	8.36	42.74
	Nicotinic acid	5.73	43.87
3000	Tap Water	7.53	42.04
	Tryptophan	8.67	46.72
	Nicotinic acid	6.12	41.50
6000	Tap Water	11.15	64.20
	Tryptophan	12.17	66.47
	Nicotinic acid	9.13	53.95
LSD ≤ 0.05		N.S	N.S

LSD = Least Significant Difference;

NS = Non-Significant.

The level of lipid peroxidation, expressed as MDA content, has been used as an indicator of free radical damage to cell membranes (Du & Wang, 2009). Shalata and Neumann (2001) reported that salt-stress increased the accumulation of lipid peroxidation products in roots, stems and leaves. Addition of ascorbic acid partially inhibited this response but did not significantly reduce Na<sup>+</sup> uptake or plasma membrane leakiness. They concluded that root treatment with exogenous ascorbic acid remarkably increased the capacity of tomato seedlings tolerance to salt stress. The increase in plant resistance to salt stress was associated with the anti-oxidant activity of ascorbic acid and a partial inhibition of salt-induced increases in lipid peroxidation by active oxygen species.

Cell membrane damage due to salt stress is associated with oxidative stress as a result of lipid peroxidation (Du

& Wang, 2009). Hussein and Orabi (2008) subjected onion plants to two levels of salinity, i.e. 3000 and 6000 ppm, and reported an increase in lipid peroxidation and electrolyte leakage with an increase in salinity. However, the salinity induced damage was mitigated by application of thiamine. Sreenivasulu et al. (1999) observed electrolyte leakage in foxtail millet was directly correlated to concentration of NaCl in the growth media.

Tuna, Kaya, Dikilitas, Butan, and Altunlu (2007) revealed that application of salicylic acid (SA) decreased the amount of ion leakage in salt stressed maize plants indicating that SA facilitated the maintenance of membrane functions under stress conditions. Similar beneficial effects of SA on reduced electrolyte leakage was reported in salt stressed tomato leaves and chilling stressed corn leaf, rice leaf, and cucumber hypocotyl (Szltveit & Kang, 2001; Stevens, Senaratna, & Sivasithamparam, 2006). Akram and Ashraf (2011) demonstrated reduced leakage of K<sup>+</sup> in sunflower plants by the application of both ascorbic acid and thiamine.

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