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ROLE OF PROLINE AND INOCULATION IN MITIGATING SALINITY STRESS OF IRRIGATION WATER AND ENHANCED GROWTH OF WHEAT PLANT.

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ABSTRACT

A pot experiment was conducted in the greenhouse of Soils and Water Research Department, Atomic Energy Authority, Egypt, in the winter season of 2011/2012. Four irrigation water differed in EC were used in the experiment: 0.25 dS m^{-1} (Fresh water as a control), and three saline irrigation water 2, 4 and 6 dSm^{-1}. Basic supplement of P and K fertilizers were applied to each pot, at rate of 200 mg kg^{-1} soil as super phosphate and 50mg kg^{-1} soil as potassium sulfate, respectively. Ammonium sulfate was added at rate of 120 mg N kg^{-1}. Proline was added at three concentrations, zero, 15 and 30 mg l^{-1} and sprayed in the early morning. Data showed that inoculation with *Azotobacter* alone led to a significant increase in dry weight of wheat plants and weight of spike compared to uninoculation, regardless of the addition of saline water. Data obtained also indicated that the increasing concentration of spraying proline acid from zero to 30 ppm in most cases lead to increase growth and dry weight of wheat crop as well as the weight of spikes. This holds true at the different levels of saline irrigation water or even added to tap water (Control). The concentration of sprayed proline (30 ppm) in most cases gave the highest value of dry matter yield of both plant and spikes. Nitrogen uptake by plants tended to decrease with increasing irrigation water salinity levels. Severe decrease was noticed with salinity level of 6 dS m^{-1}. Gradual decrease in nitrogen uptake by plants was detected with 2 and 4 dS m^{-1} salinity levels as compared to control treatment. The adverse effect of salinity levels of irrigation water was slow down by proline additions. Enhancement of nitrogen uptake was induced by addition of proline at 30 ppm concentration, followed by those of 15 ppm concentration.

Keywords: Azotobacter, Proline, Saline water, Wheat (*Triticum aestivum* L.)

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the world's major cereal crops. It is grown to meet the food demand of over growing population of the worlds. Growth of wheat seedlings (*Triticum aestivum* L.), like other crops, is negatively affected by salinity stresses (Khan, and Gul 2006). Salinity is the major abiotic factor limiting plant growth and productivity (Allakhverdiev et al. 2000); (Soltania et al. 2006); (Munns & Tester, 2008; Manisha jan et al 2013).

Salinization affects many irrigated areas mainly due to the use of brackish water. Worldwide, more than 45 million hectares of irrigated land have been damaged by salt, and 1.5 million hectares are taken out of production each year as a result of high salinity levels in the soil (Munns & Tester, 2008). High salinity affects plants in several ways: water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, genotoxicity (Hasegawa, Bressan, Zhu, & Bohnert, 2000; R.

Munns, 2002; Zhu, 2007). Together, these effects reduce plant growth, development and survival.

Plants have defense mechanisms that allow them to acclimatize in saline environment. One of them is the accumulation of certain organic metabolites/osmolytes. These are also collectively known as compatible solutes (Serraj and Sinclair2002; Ashraf and Harris 2004; Vinocur and Altman 2005). Proline and quaternary ammonium compounds are key osmolytes, which help plants to maintain the cell turgor (Huang et al 2000). Proline which is usually considered as an osmoprotection agent is also known to be involved in reducing the oxidative damage by scavenging and/or reducing the free radicals. Proline accumulation was proposed to be associated with tolerance to osmotic and saline stress (Aziz and Larher 1995;Aziz et al 1999;Mansour2000). Exogenous application of proline is known to induce abiotic stress tolerance in plants (Ashraf and Foolad 2007). The amount of proline usually increases under salinity (Khatkar and kuhad 2000). During osmotic adjustment, many plants accumulate proline in response to salt stress widely believed to function as a protector against salt damage (Wang et al 2007).

Lutts, *et al.* (1996) when studied the effects of salinity stress on the aggregation of proline in 5 varieties of rice: sensitive, semi suffering and suffering, he concluded that salinity induced considerable rise in root proline content of sensitive varieties to salt, but there was no rise in root proline content of suffering varieties. After one week stress in every amount of NaCl, proline aggregated in the stem of sensitive varieties to salt but in suffering types, it aggregated just in the highest concentration of salt (40-50mM). After 2 week of tension, proline levels have been increased in all varieties but it was lesser in suffering types to salt. Regarding to quantity, exact action of proline is inconsiderable in osmotic regulation. Even if we consider that proline aggregates in cytoplasm is stead of vacuole, with this supposal that cytoplasm occupy 10% of cell volume, portion of proline in osmotic potential is variable between 1.5%-4.8% (Lutts et al. 1996). Khodary (1992) reported that proline and carbohydrate content increase in wheat and sorghum plant lets by increase of NaCl while amino acids' content decreases (Khodary, 1992).Tombesi (1986) studied the effect of drought tension on the photosynthesis, stomata's status and carbohydrates' amount in olive plants. They observed that leaf dissolved saccharin amount increases by decreasing the water of soil but leaf amylum amount decreases in a liner manner (Tombesi et al. 1986).

Wheat is glycophytes, which are sensitive to even low salt concentrations (Manisha jan et al; 2013). Proline appears to be the preferred organic osmotic compound in many plants. The proposed functions of proline under stress conditions include osmotic adjustments, protection of enzymes and membrane, as a hydroxyl radical scavenger, as well as acting as a reserve of energy and nitrogen for utilization during exposure to salinity.

This work aimed at study the effect of salinity, inoculation and amino acid proline on growth and yield of wheat.

MATERIALS AND METHODS

A pot experiment was conducted in the greenhouse of Soils and Water Research Department, Nuclear Research Center, Atomic Energy Authority, Egypt, in the winter season of 2011/2012. The soil tested through this study was collected from Inshas, Sharkia Governorate from (0-15cm depth) layer. The Soil samples were air dried, ground and sieved to pass through a 2 mm sieve then subjected to physical and chemical analysis (Table 1)

Four irrigation water differed in EC were used in the experiment: 0.25 dSm^{-1} (Fresh water as a control), and three saline irrigation water 2, 4 and 6 dSm^{-1}, which prepared by mixing fresh water (0.98 dSm^{-1}) with sea water (48.0 dSm^{-1}) at certain ratios according (Ayers and Westcott, 1989) using the following equation:

[$EC_{(sea\ water)}$ X Proportion used]+ [$EC_{(fresh\ water)}$ x proportion used = $E_{(mix\ water)}$]

Table (1): Some physical and chemical properties of experimental soil.

Coarse sand %	Fine sand %	Silt %	Clay %	Texture	Cations (M eq 100 gm^{-1} soil)				Anion (M eq 100 gm^{-1} soil)			
					Ca	Mg	Na	K	CO_3	HCO_3	Cl	$SO_4^=$
64.1	26.4	2.7	6.8	Sandy	1.25	1	0.32	0.09	0.0	0.88	1.25	0.53

pH	EC(dSm^{-1})	O.C%	O.M	T.N	C/N ratio	$CaCO_3$%
7.97	0.27	0.017	0.03	0.007	2.43	1.0

Soil suspension 1:2.5 soil: water

Grains of wheat cultivar (*Triticum aestivum* L. cv. Saka93), supplied by the Agriculture Research center (ARC), Giza, Egypt was used. Plastic pots with 30cm in diameters and 40cm in height were uniformly packed with portions of air-dried and screened soil (10kg pot^{-1}). Basic supplement of P and K fertilizers were applied to each pot, at the rate of 200 mg kg^{-1} soil as super phosphate and 50mg kg^{-1} soil as potassium sulfate, respectively. Ammonium sulfate (20.5%N) was added at rate of 120 mg N kg^{-1} (about 580kg ammonium sulfate/fed) at two equal doses, the first one was add after two weeks from planting and the second two weeks later. Afterwards the soil was slightly irrigated by about 60% of water holding capacity (WHC). Each pot was sown with 15 wheat grains, thinned to 8 seedlings after 14 days from planting . Irrigation with diluted sea water in different concentrations was started three weeks after sowing. Proline was added at three concentrations, zero, 15 and 30 mg L^{-1} then the preparation of different concentrations of proline take place . Proline was sprayed in the early morning at rate of 15 and 30 ppm after 5 weeks of sowing. and, then sprayed in the early morning and repeated every two weeks.

The experiment includes 4 levels of salinity, two treatments of inoculation with/without *Azotobacter chroococcum* in combination with three levels of proline.It consists 24 treatments in 3 replicates. All treatments were arranged in completely randomized block design.

Wheat plants were sampled at the harvest time (after 5 months from sowing) and tillers were splitted into straw and spike for recording traits:

- Dry weights of whole plants, total nitrogen, phosphorus and potassium were estimated.
- Chemical and physical properties of soil and water as well as chemical analysis of plant were carried out according to Page et al (1982).

Statistical analysis

Data were statistically analyzed, using Statistical software program (PC-Mstat) according to Power (1985). The values of least significant difference (L.S.D) were calculated according to Gomez & Gomez (1984).

RESULTS AND DISCUSSION

Data in Tables (2, 3) showed that inoculation with *Azotobacter chroococcum* alone led to a significant increase in dry weight of wheat plants and weight of spike compared to uninoculation, regardless of the addition of saline water. This may be attributed to the production of stimulant materials to growth, such as Gibberellins, Auxins, Cytokines and Vitamins that increase the size of the root system and growth of root hairs and then lead to increased nitrogen content of the plants which enhanced growth (Papic-vidakovic, 2000).

Table (2): Effect of water salinity, inoculation, proline acid and interaction between them on the dry weight of wheat plant g pot^{-1}

Water salinity dSm^{-1}	Un-inoculation				Inoculation			
	Proline acid(ppm)							
	0	15	30	Mean	0	15	30	Mean
0	14.5 ef	16.44 d	19.56 C	16.71	17.30 d	22.41 b	25.65 a	21.78
2	10.10 hij	12.95 efg	13.61 Ef	12.22	12.85 efg	16.55 d	19.60 c	16.33
4	9.23 ij	11.10 ghi	11.15 Ghi	10.49	12.00 fgh	14.10 ef	14.35 e	13.48
6	6.10 k	9.90 hij	9.95 Hij	8.65	8.25 j	10.35 hij	12.65 efg	10.41
	9.73	12.59	13.56		12.6	21.13	18.07	

L.S.D = 2.051
At 0.05 level

Table (3): Effect of water salinity, inoculation, proline acid and interaction among them on the weight of spike of wheat plant (g pot^{-1})

Water salinity dSm^{-1}	**Un-inoculation**				**Inoculation (Azotobacter chroococcum)**			
	Proline acid(ppm)							
	0	15	30	Mean	0	15	30	Mean
0	4.80 bcde	5.05 bcde	5.80 Bc	5.21	5.13 bcde	7.85 a	8.15 a	7.04
2	4.15 cdef	4.45 bcde	5.05 Bcde	4.55	4.65 bcde	6.00 b	7.70 a	6.11
4	3.00 fg	4.15 cdef	4.80 Bcde	3.98	4.16 cdef	4.96 cde	5.62 bcd	4.91
6	2.81 g	3.0 fg	3.51 Efg	3.10	3.50 efg	4.00 efg	4.85 bcde	4.11
Mean	3.69	4.16	4.79		4.36	5.70	6.58	

L.S.D = 1.562

Data obtained also indicated that the increasing concentrations of spraying proline acid from zero to 30 ppm in most cases lead to increased growth and dry weight of wheat crop as well as the weight of spikes. This holds true at the different levels of saline irrigation water or even added to tap water (Control). The concentration of sprayed proline (30 ppm) in most cases gave the highest value of dry matter yield of both whole plant and spikes. In this respect, proline acid could be used as a potential growth regulator to improve salinity stress resistance in several plant species (Sheteawi, 2007), as well as proline and quaternary ammonium compounds are key osmolytes, which help plants to maintain the cell turgor (Huang et al.,2000). In addition, bacterial inoculation led to significant increase in dry weight of the plant and also wheat spikes for all levels of saline irrigation water compared to the non-inoculation treatment where the relative increase was (30.34%, 33.38%, 28.50%, 20.34%),(35.12%, 34.28%, 23.36%, 32.58%) for plant dry weight and spikes, respectively, at zero, 2, 4, 6 dSm^{-1} over the control. The highest value was recorded after inoculation with saline water (2 dSm^{-1}). Also, Azotobacter is classified as one of the Rhizospheric bacteria (plant growth promoting rhizobacteria), which have the ability to produce exopolysaccharides that constrained sodium cation, as well as reduces the availability of sodium to absorption by the plant which helps to reduce the adverse effect of salt stress in the plants. Some scientists also found that Azotobacter produce wide spectrum of polysaccharides differ in their direct impact on ions in the plant depending on the type of ion (Ashraf and Harris, 2004).

According to Johari-Pireivatlou (2010), seed yield, straw yield and 1000 kernel weight of different wheat genotypes were decreased by water stress, compared with control. It have been widely re- ported that plant yield decreased under water stress (Tatar and Gevrek, 2008; Kameli and Losel, 1996). In the same direction, Galal et al. (2012) indicated that the dry matter yield accumulated by stalks and roots of sorghum was enhanced by addition of organic sources combined with either 20 mg or 40 mg of proline acid as compared to plants only treated with mineral fertilizer. Increasing water salinity inhibited the dry matter accumulation.

In the same way, Tammam et al. (2008) reported that shoots and spikes dry matters were either unchanged or even stimulated to increase toward 180 mM NaCl then a quick reduction was observed. They assumed that it is associated with the increase of leaf area and photosynthetic pigments up to 180 mM NaCl. On line, applied NaCl significantly decreased dry mass of maize plants (Turan et al. 2009), as well wheat plants (Turan et al. 2007).

Nitrogen uptake

Nitrogen uptake by plants tended to decrease with increasing irrigation water salinity levels. Severe decrease was noticed with salinity level of 6 dS m^{-1}. Gradual decrease in nitrogen uptake by plants was detected with 2 and 4 dS m^{-1} salinity levels comparing to the control treatment. The adverse effect of salinity level of irrigation water was slow down by proline additions. Enhancement of nitrogen uptake was induced by addition of 30 ppm concentration followed by those of 15 ppm concentration. Similar trends, but to somewhat high extent were detected with the inoculated plants as compared to the uninoculated ones.

Table (4): Effect of water salinity, inoculation, proline acid and interaction among them on nitrogen uptake by wheat plant (mg $plant^{-1}$)

Water salinity dSm^{-1}	Un-inoculation				Inoculation (Azotobacter chroococcum)			
	Proline acid(ppm)							
	0	15	30	Mean	0	15	30	Mean
0	8.26 cfg	8.93 def	9.35 bcde	8.85	10.90 bc	11.00 b	14.50 A	12.13
2	7.60 efgh	8.16 efg	8.75 def	8.17	9.15 cde	10.28 bcd	13.60 A	11.01
4	5.13 ij	6.33hi	6.90 gh	6.12	7.35 fgh	8.61 defg	10.83 Bc	8.93
6	1.65 l	1.96 l	2.66 kl	2.09	2.37 kl	3.93 jk	4.71 ij	3.67
Mean	5.66	6.34	6.91		7.44	8.45	10.91	

L.S.D = 1.663

On the basis of findings of Talat *et al.* (2013) it can be concluded that salt stress negatively affects the growth, morphology and physiology of wheat but the exogenous application of proline significantly ameliorates the harmful effects of salt. They found that 100mM proline application was effective than 50mM produce the more positive results. In general, the proline induces the salt tolerance in both two Pakistani cultivars of wheat. In a green house experiment, Galal *et al.* (2012) using sorghum as a test crop revealed that N uptake by stalks and roots was severely declined when exposed to 3 and 6

dSm^{-1} water salinity levels. Sever reduction in N uptake by plant was noticed with 6 dSm^{-1} salinity level. Organic additives significantly increased the nitrogen uptake by plants over those recorded with plants only fertilized with mineral form. Foliar application of 20 mg of proline acid induced higher N uptake than those resulted from spraying 40 mg of proline acid. It was more pronounced under the high salinity level of 6 dSm^{-1}.

Exogenous application of proline was found to be useful in alleviating the shocking effects of salt stress on seed germination of different wheat genotypes (Khan *et al.* 2006). This decrease in growth due to a reason of too much accumulation of Na^+ (McConnell et al. 2008).

Earlier work done by the author and co-workers using ^{15}N isotope (Gadalla *et al.* 2007) indicated a slight increase in N uptake by maize with increasing salinity level of irrigation water up to 6 dS m^{-1} but it relates to nitrogen application rates.

Phosphorus uptake

As shown in Table (5), P uptake by uninoculated wheat plants severely decreased with high level of salinity (6 dS m^{-1}) and gradually decreased with 4 dS m^{-1} while there was no significant difference between 2 dS m^{-1} and control. Sprayed proline acid induced a little bit improvement of P uptake especially at 30 ppm concentration. This holds true with all water salinity levels. Bacterial inoculation, in general improved P uptake by plants either irrigated with tape water or saline one. Similar effects of salinity and proline applications were detected.

Table (5): Effect of water salinity, inoculation, proline acid and interaction among them on phosphorus uptake in wheat plant (mg $plant^{-1}$)

Water salinity dSm^{-1}	Un-inoculation				Inoculation (Azotobacter chroococcum)			
	Proline acid(ppm)							
	0	15	30	Mean	0	15	30	Mean
0	1.55 abcdef	1.73 abcde	1.80 abcde	1.69	2.10 abc	2.25 abc	2.39 a	2.24
2	1.66 abcdef	1.75 abcde	1.88 abcd	1.76	1.93 abcd	2.30 ab	2.30 ab	2.17
4	0.95 efgh	1.43 cdef	1.69 abcde	1.35	1.22 defg	1.49 bcdef	1.95 abcd	1.55
6	0.25 H	0.33 h	0.54 gh	0.37	0.43 gh	0.56 gh	0.88 fgh	0.62
Mean	1.10	1.31	1.47		1.42	1.65	1.88	

L.S.D. = 0.0140

In this regard, our results are in contrast with Faramawi and Abd El-Hamid (2012) where they indicated that nitrogen and phosphorus content of mangrove seedlings increased significantly with increasing salinity levels, but we are in agreement with them concerning the enhancement effect of biofertilizers e.g. Azotobacter, Azospirillum, Phosphate dissolving bacteria (P.D.B.), mycorrhizae as a single or as a mixture inoculation. They added that the highest significant increase recorded with mixed inoculation and was increased as salinity level increased for individual or mixture inoculation.

On the other hand, Galal *et al.* (2012) clarified that the effect of water salinity on P percent in sorghum organs was not clear cut evident. Similarly, the difference between proline concentrations was not remarkable enough.

Sodium uptake

Sodium uptake by wheat plant is presented in Table (6). It is obvious that sodium content in plants was increased with increasing water salinity levels. These are true either plants inoculated or uninoculated with bacterial strain. It was clear that both inoculation and exogenous proline addition has no significant positive effect on sodium content in plants.

These results are in harmony with those of Sadat Noori et al. (2010) who found that salinity had positive significant effect on Na^+ where it increases with increasing salinity levels. Also, Turan et al. (2009) found that NaCl tend to increase Na and Cl concentrations of maize plant, and wheat cultivar (Turan et al. 2007), but they detected decreases in N and K uptake by wheat cultivar with increasing salinity.

Similarly, Tammam *et al.* (2008) found that sodium content increased significantly in the three organ (shoot, root and spikes) of wheat but the percent of increase was varied considerably among the three organs especially at sever salinity and the opposite pattern was observed in the accumulation and distribution of K^+ , Ca^{+2} and Mg^{2+}. K^+ / Na^+ ratio decreased in response to salt stress in root, shoot and spikes.

Table (6): Effect of water salinity, inoculation, proline acid and interaction among them on sodium uptake in wheat plant (mg plant^{-1})

Water salinity dSm^{-1}	Un-inoculation				Inoculation (Azotobacter chroococcum)			
	Proline acid(ppm)							
	0	15	30	Mean	0	15	30	Mean
0	0.41 c	0.50 abc	0.50 abc	0.47	0.49 Bc	0.54 abc	0.60 abc	0.54
2	0.49 abc	0.41 c	0.55 abc	0.48	0.57 abc	0.59 abc	0.70 abc	0.62
4	0.52 abc	0.45 bc	0.56 abc	0.51	0.61 abc	0.65 abc	0.72 abc	0.66
6	0.73 abc	0.55 abc	0.67 abc	0.65	0.82 a	0.69 abc	0.78 ab	0.76
Mean	0.53	0.47	0.57		0.62	0.61	0.70	

L.S.D= 0.3460

Exogenous application of compatible solutes e.g. proline or glycine betaine may reduce stomatal opening and reduce transpiration rate (Raghavendra *et al.,*1987). Sodium concentration increased in both wheat cultivars but K concentration decreased in both wheat cultivars, however exogenous application of proline. These results similar to the earlier findings which were reported by Patil *et al.* (1996). There are various reasons for these results. Jescke *et al.* (1998) suggested that the association of induced increase in plant Na^+ with a decrease in K^+ content may be due to the competition for sites through which influx of both ions occurs.

Also, we are in consistent with Hussein *et al.* (2011) who stated that increasing salt concentration from 3000 to 6000 ppm decreased the all growth and yield parameters such as plant height, number of leaves, dry weight of stem, leaves and spikes. Spraying ascorbic acid (ASA) improved the parameters of growth and yield and decreased the salt effect. Increasing the ascorbic acid spraying rate from 0 to 200 ppm increased the uptake of essential nutrients of wheat but decreased the Na and Cl uptake, so the ascorbic acid plays an important role for decreasing effects of saline water.

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دور البرولين والتلقيح في التخفيف من الاجهاد الملحى لمياه الرى وتحسين نمو نبات القمح

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أقيمت تجربة اصص فى الصوبة الزراعية بهدف تقييم دور البرولين والتلقيح فى التخفيف من الاجهاد الملحى لمياه الرى وتحسين نمو نباتات القمح ولقد تم اضافة البرولين بمعدلات صفر ، ١٥، ٣٠ ppm وكذلك مياه الرى المالحة بمعدلات صفر، ٢,٤,٦ ديسسيمنز وفى وجود او عدم وجود التلقيح والذى تم باضافة الازوتوباكتر.أوضحت النتائج أن التلقيح مع الآزوتوباكتر منفردة أدت إلى زيادة كبيرة في الوزن الجاف لنباتات القمح والسنابل مقارنة بالكنترول غير الملقح، بغض النظر عن إضافة المياه المالحة .وأشارت البيانات التي تم الحصول عليها أيضا أن زيادة تركيزات حمض البرولين رشا من صفر إلى 30ppm في معظم الحالات تؤدي إلى زيادة النمو والوزن الجاف للمحصول القمح وكذلك وزن السنابل. و ينطبق هذا على المعدلات المختلفة من مياه الري المالحة أو حتى الماء العذب (الكنترول) . أعطى تركيز البرولين رشا على الاوراق بمعدل 30ppm في معظم الحالات أعلى قيمة لعائد المادة الجافة في كل من النبات والسنابل. امتصاص النيتروجين من النباتات يميل إلى الانخفاض مع زيادة مستويات ملوحة مياه الري . وقد لوحظ الانخفاض الشديد مع مستوى ملوحة 6 ديسيسيمنز. ولقد ظهر الانخفاض التدريجي في امتصاص النيتروجين بواسطة النباتات مع تركيز ٢ و ٤ ديسيسيمنز- مقارنة مع معاملة الكنترول . وكان التأثير السلبي لمستويات ملوحة مياه الري عند عدم اضافة البرولين..هذا مما يوضح أثر البرولين فى تقليل الاثر الضار للملوحة. وهذا يوضح زيادة امتصاص النيتروجين بإضافة البرولين بمعدل 30 جزء في المليون يليه 15 جزء في المليون .

قام بتحكيم البحث

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