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Effect of Proline on Resistance of Potato Crop (*Solanum tuberosum* L.) for the Negative Effects of Water Irrigation Salinity

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ABSTRACT

Two field experiments were conducted during the season of 2013/2014 and 2014/1015 at Sabaheya Soil, Water and Environment Research Institute, Agricultural Research Station to investigate the role of proline foliar application (0 ,150 and 300 ppm) in counteracting the harmful effect of salinity stress at various concentartions (500,1000,2000 and 3000ppm) on potato plants growth characters, yield and its components. The results showed that all growth characters including: plant length, number of shoot, total yield of tuber/plant (gm), number of tubers/plant and average tuber weight/plant were decreased with increasing salinity stress level alone or combined with applied proline . On the other hand, salinity stress levels alone or combine with applied proline foliar application increased tuber dry matter%, starch content, total sugars and reducing sugars. It could be noticed that applied proline could partially reduced the harmful effect of salinity stress on growth and yield characters of potatoes.

Key words: Potatoes; proline; salinity stress

Introduction

Accumulation of salts in soil and water irrigation have a negative effect on the production of a wide variety of crops (Rinse *et al*., 2013). Also they added that for various reasons the area of salt affected soils will rapidly expand in the near future. As the world population continues to grow, the availability of renewable fresh water resources for agriculture will decrease, and simultaneously the area of irrigated land will increase in the attempt to satisfy the need for more food. There are relatively few studies that describe which traits remained in current potatoes cultivars that might be a starting point to improve tolerance of potato crop to salt strees. Drought problems in arid and semi-arid regions are forcing to use marginal quality waters (brackish, reclaimed, drainage and waste water) in irrigation. Many countries over the world are planning to use those waters in their long-term development plants (Cart Zoulakis *et.al*., 2005). About 17% of the global cultivated area is irrigated and more than 30% of the agricultural production comes out of this area (Hillel, 2000). Taking into consideration that global salt affected soils are 830 million ha., it is obvious that saline water- yield relations should be investigated in more detailed studies (Martinez and Manzur, 2005).The studies (Bruns and Caesar, 1990, Levy and Tai, 2013, Qadir *et al*.,2010) conducted using saline water showed that saline water is changing soil physical and chemical properties as a result of accumulated salt content.
The increase of salt content in soil also causes hormonal changes (Munns, 2002), decay in carbohydrate metabolism (Gao *et al.*, 1998), decrease in certain enzyme activities (Munns, 1993) and close up stomata and decrease transpiration rate and yield (Ben Asher *et al*., 2006).

Potato is the fourth most cultivated crop world-wide and in semi arid areas where salt stress is a serious problem, productivity is considerably reduced .Wild potatoes growing under harsh conditions are relatively stress tolerant, but extensive breeding and selection for traits other than a biotic stress tolerance have resulted in cultivars that are considered moderately salt tolerant (FAO, 2010). Potato is known as salt sensitive crop (Mass and Hoffman, 1977) especially early development stage. Plant height, leaf area and fresh weight accumulation were decreasing depending on increased salinity (Heuer and Nadler , 1995). Physiological , potato crop is more sensitive to salinity early in the tuber formation (Bruns and Caesar,1990).
Crops are accumulating proline as a first physiological reaction when they are exposed to stress factors such as salinity and drought. Increase of proline concentration in the vacuole inside the cell is a measure of how long the crop is under stress and how the crop is tolerant to that stress factor. Researches indicated that proline is

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occurred during protein decay resulting and is synthesized inside the cell. It is reported that proline has significant function in stabilizing osmotic effects by balancing of ion concentrations such as Na, K, Mg, and Ca , in strengthening the cell wall and in other enzymatic actions (Iba,2002). Similarly, as a result of higher Na concentration, proline, which is a stress protein is produced and accumulated in the cells(Ayers and Westcot ,1985).
Therefore, the objectives of this study were:
1- Determining the effect of salinity stress on growth, yield and chemical components of potato crop.
2- Studying the effectiveness of the amino acid proline; as foliar application, in resistance the adverse effect of water irrigation salinity.

Materials and Methods

A pot experiment was carried out during the two successive winter seasons of 2013/2014 and 2014 /2015 at Sabaheya Soil, Water and Environment Research Institute to investigate the effect of NaCl salinity on potato plant growth , yield and it's component , in addition to study the possibility of using proline foliar application for controlling the harmful effects of water irrigation salinity.

The experimental soil was sampled twice: the first one at the beginning of the growing season, the second one at the end of the growing season and analyzed for some physical and chemical properties according to the methods outlined in Page *et al.* (1982), Gee and Bauder (1986) and Shainberg(1990) as shown in Table (1 and 2).

Table 1: Initial physical and chemical properties of the investigated soil

Saturated soil extract	**Alluvial soil**
pH	8.16
ECe, dS/m	2.48
CaCO3, %	2.54
Organic matter, %	1.21
CEC, cmolc / kg	26.3
Soluble cations, (meqL^{-1})	
Ca++	6.54
Mg++	6.32
Na+	14.60
K+	0.32
Soluble anions , (meqL^{-1})	
HCO3–	5.88
Cl^-	17.06
SO_4^{--}	4.84
ESP, %	7.88
Particle size distribution, (%)	
Sand	53.03
Silt	13.92
Clay	33.05
Soil texture	Sandy clay loam

Table 2 : Average composition of saturated soil extract at the end of the experiment.

Treat. EC_{iw} (ppm)	EC_e dS/m	pH	Soluble cations, meq/L				Soluble anions, meq/L			ESP,%
			Ca^{2+}	Mg^{2+}	Na^+	K^+	Cl-	HCO_3^-	SO_4^{-2}	
500	2.02	8.14	7.6	5.3	10.4	0.12	6.9	7.1	6.8	6.2
1000	2.65	8.27	6.2	5.9	15.8	0.14	12.4	8.7	6.9	8.6
2000	4.76	8.35	7.2	6.8	31.9	0.16	30.9	8.8	6.3	14.4
3000	6.63	8.39	7.4	5.7	42.5	0.14	41.3	8.9	5.5	19.1

** ESP = Exchangeable sodium percentage*
Tap water = 500 ppm

Potato (Solanum tuberosum; cv. Carozo) were obtained from Chipsy Co., Nubarya region, Behira Governorate, Egypt. Natural salt crust was used as a source of salinity in this experiment at different concentrations 500(tap water), 1000, 2000 and 3000ppm. The L-proline was obtained from Lopa Chemical Co. Plastic pots of 40 cm diameter were used in these experiments; each pot was filled with 10 kg sandy soil.

Treatments:

Pots were divided into four groups consisted of 60 pots for each. Phosphorous as calcium super phosphate was added as recommended dose before planting. The potassium fertilizers as potassium sulphate was added at 0.6 g/ pots and the nitrogen fertilizer as ammonium nitrate at 1.5 g/ pots were mixed and add by weekly from age 21 days till age 100 days. One tuber/pot was planting on 15th September. After 21 days from planting the pots of the first group were watered with tap water (500 ppm) (control) while, the other groups watered with saline solution at the levels 1000, 2000, and 3000 ppm. The irrigation water was applied every 7 days. Pots were divided into three sub groups "20 pots for each". Plants were sprayed with proline at 0, 150, 300 ppm every 7 days with salinity treatment. Harvesting was carried out after 120 days from planting in both seasons.

Measurements of vegetative growth and yield characters:

Samples of nine random plants from each treatment were determined the vegetative growth characters plant length and number of branches after 80 days of planting. After 120 days of planting, nine random plants from each treatment were used for determining number of tuber/plant, total yield of tuber / plant (g) and average tuber weight / plant (g).

Tuber root quality:

Percentage of tuber's dry y matter% was determined in tuber's slices, by weighing a certain weight of fresh tubers and then oven dried at 70^0 C for 48 h.

Percentage of total sugars and reducing sugars of the tubers were determined using 5 g of fresh tuber root, using sulphuric acid and phenol (5%), then they colourimetrically determined , according to the method described in (Dubios *et al*., 1956).

Tuber root starch content (%) was determined colourimetrically using a sample of 1 g of fresh tuber, according to the method described in A.O.A.C. (1970).

Experimental design and statistical analysis:

The experimental layout was arranged in two way: factorial in a randomized complete design (C.R.D.) with three replicates. The collected data of the experiments were combined and analyzed using combine analysis of variance. Comparisons among the means of different treatments were carried out using Duncan,s(1995) Multiple Range test procedure at p=0.05 level of significant, as outlined by Snedecor and Cochran (1980).

Result and Discussion

Vegetative characters and tuber root quality:

Data presented in Table (3) showed that all growth characters including plant length, number of shoot, total yield of tuber/ plant (gm.) and number of tuber/ plant were significantly decreased with salinity stress levels alone or combined with proline in both seasons , while there was insignificant effect for characteristic average tuber weight/plant(gm.) in second season. The great reduction in these parameters was observed under high salinity level (3000ppm.). Applied proline foliar application enhanced the plant to tolerate the harmful effect of high salinity level. Similar results were reported by (Khattab and Afifi, 2009) who found that all growth characters including stem, yield and its component of canola were significantly decreased with increasing salinity levels. Sakr *et al*., (2007) reported that proline accumulation is one of the most frequently reported modifications induced by water deficit and salt stress resistance mechanisms . Arteca (1996) reported that the primitive effect of proline on stem and root length may be the result of increasing cell division in the apical meristems, or increased cell division and cell enlargement. . Moreover, Mathur and Wattal (1995) on Canadian rape found that salinity stress reduced plant hight and shoot dry weight. Sakr *et al*., (2007) stated that salinity affected all stages of Canola growth and development as well as yield of Canola plants. The yield was much more depressed by salt than the vegetative growth which may be attributed to a decreased in the viability of pollen grain or in the receptivity of stigmatic surface or both.The inhibitory effect of salinity on potato growth in the present investigation may be due to decreasing meter sematic activity and/ or cell enlargement (Al-Hamdany and Mohammed 2014), damaging growth cells so that they cannot perform their function, limiting their supply of essential metabolites and perturbing the functioning of vital components of photosynthesis Yang and Britton (1990).

Tuber quality characteristic:

Data in Table (4) showed that dry matter %, total sugar, reducing sugar and starch content were significantly increasing with increasing salinity levels alone or combined with applied proline foliar application. The great value of reducing sugar was observed under salinity at 3000ppm, in combination with proline at 300 ppm in both seasons. While, the great value of total sugars were observed under high salinity levels (3000 ppm.).Khattab and Afifi (2009) found that salinity stress levels (6000 or 9000 ppm) alone or combined with applied proline increased soluble sugars. . Ashraf and Sharif (1998) stated that application of proline alleviate the harmful effect of salinity stress on growth and biochemical constituents. Munns and termaat(1986) reported that the accumulation of non reducing sugars was the result of an enhanced efficiency in the use of carbon coupled to a reduction in cellular metabolism that could favor the accumulation of respiratory substrate to support the osmotic adjustment required to survive in saline media. In addition to , the foliar application of proline on tobaccoe (Agboma,*et al*.,1996) , maize , sorghum , wheat and wild Hordeum (Garthwaite *et al.*, 2005) and potato (Al-Hamdany and Mohammed 2014), enhance leaf area, dry matter accumulation and finally plant growth as well as increased soluble sugars as increased in proline treated discus. Bekant and Mehmet (2014) reported that irrigation water salinity reduced tuber initiation and tuber bulking also, proline play important role for minimize the harmful effect of salinity:

1-It improves the stability of some cytoplasmic and mitochondrial enzymes.

2-increased the salvation of protein

3-Proline plays an important role in the protection of enzymes or membranes against salinity (Ozdemir, *et al*, 2004).

Table 3: Effect of proline and salinity and their interactions on Vegetative characters and tuber root quality of potato plants grown in 2013/2014 and 2015 growing seasons.

Character Treatments	**Plant length(cm)**					**No. of shoot**				
	Salinity level(ppm)					**Salinity level(ppm)**				
					2013-2014					
Proline	**500**	**1000**	**2000**	**3000**	**Mean**	**500**	**1000**	**2000**	**3000**	**Mean**
Zero	52.5abc	47cd	44.5de	35.5f	44.88B	4ab	3ab	3ab	2.33b	3.08A
150 ppm	57.33ab	50.5bcd	46.33cd	37.5ef	47.92AB	4.33a	3.67ab	3.33ab	3ab	3.58AB
300 ppm	59.67a	49.67cd	45cd	38.5ef	48.21A	4.67a	4ab	4ab	3.67ab	4.09B
Mean	56.5A	49.06B	45.28B	37.17C		4.33A	3.56AB	3.44AB	3B	
					2014-2015					
Zero	61.67a	57.33ab	56.67ab	49b	56.17A	4.33abcd	3.67cd	4bcd	3.33d	3.83B
150 ppm	61.67a	58.5ab	57ab	56ab	58.29A	5.33abc	5abcd	4.67abcd	4.33abcd	4.83A
300 ppm	65.33a	61.67a	57.33	56.67ab	60.25A	6a	5.67ab	5abcd	4.67abcd	5.34A
Mean	62.89A	59.17AB	57BC	53.89C		5.22A	4.78AB	4.56AB	4.11B	
Character	**Total yield of tuber/plant(g)**					**No. of tuber/plant**				
					2013-2014					
Zero	711a	648bc	591d	541e	MEAN	5.67ab	4.67bcd	4.67bcd	4d	4.75B
150 ppm	708.33a	680ab	633.67bcd	610cd	622.75B	6a	6a	5.33ab	4.67bcd	5.5A
300 ppm	681.67ab	670ab	640bcd	589d	658A	6a	6a	5.33abc	4.33cd	5.42A
Mean	700.33A	6666B	621.56C	580D	645.17AB	5.89A	5.56AB	5.11B	4.33C	
					2014-2015					
Zero	745a	649bcd	583ef	549f		6ab	5.33abc	5.33abc	4.33c	5.25B
150 ppm	730a	695ab	627cde	615de	631.5B	6a	6.67a	5.67abc	5.33abc	5.92A
300 ppm	739a	689abc	636bcde	617de	666.75A	6.33ab	6ab	5.67abc	5bc	5.75AB
Mean	738A	677.67B	615.33C	593.67C		6.11A	6A	5.56AB	4.89B	Mean
	Average tuber weight /plant (g)									
			2013-2014							
Zero	126.3ab	140.8a	127.65ab	135.25ab	132.5A					
150 ppm	112.1ab	114.169ab	112.87ab	131.67ab	117.7B					
300 ppm	110.52b	111.67ab	120.96ab	137.63ab	120.19AB					
Mean	116.31B	122.21AB	120.49AB	134.85A						
			2014-2015							
Zero	126.48a	122.7a	110.09a	128a	121.82A					
150 ppm	109.93a	104.92a	110.67a	116a11	110.38A					
300 ppm	119.15a	116.33a	113.06a	123.4a	117.99A					
Mean	118.52A	114.65A	111.27A	122.47A						

Table 4: Effect of proline and salinity and their interactions on tuber quality characteristic of potato plants grown in 2013/2014 and 2014/2015 growing seasons.

Character Treatments	**Tuber dry matter%**					**Total sugars**				
	Salinity level(ppm)					**Salinity level(ppm)**				
					2013-2014					
Proline	**500**	**1000**	**2000**	**3000**	**Mean**	**500**	**1000**	**2000**	**3000**	**Mean**
Zero	17.27e	19.73c	22.04b	23.93a	20.74A	6.74cd	7.13bc	7.29abc	8.33a	7.37A
150 ppm	17.95de	19.82c	22.04b	23.94a	20.94A	5.75de	6.85c	7.26abc	8.24ab	7.03A
300 ppm	18.03d	20.08c	22.16b	23.98a	21.08A	5.61e	6.80c	7.19abc	7.61abc	6.8A
Mean	17.75D	19.88C	22.08B	23.95A	20.74A	6.03C	6.93B	7.25B	8.06A	
					2014-2015					
Zero	17.98e	19.2d	22.55c	23.63a	20.84A	6.65d	7.48bc	7.71abc	8.25a	7.52A
150 ppm	18.05e	19.5d	22.01bc	23.68a	20.81A	5.99e	7.14cd	7.54bc	8.05ab	7.18B
300 ppm	18.33e	19.55d	22.15b	23.9a	20.98A	4.81f	7.27c	7.61bc	7.66bc	6.84C
Mean	18.12D	19.42C	22.24B	23.74A		5.82D	7.29C	7.62B	7.99A	
	Reducing sugars					**Starch Content**				
					2013-2014					
Zero	3.02d	3.62c	3.97bc	4.21abc	3.71	14.12f	15.17de	15.75cd	18.11a	15.79
150 ppm	2.98d	4.18abc	4.31ab	4.47ab	3.99	14.87def	15.77de	16.77bc	18.37a	16.45
300 ppm	2.78d	4.13abc	4.43ab	4.62a	3.99	14.57ef	16.7bc	17.1b	18.67a	16.76
Mean	2.93	3.98	4.24	4.43		14.52	15.88	16.54	18.38	
					2014-2015					
Zero	2.81d	4.04b	4.04b	4.22ab	3.78B	14.17f	15.27e	15.73de	18.33a	15.88B
150 ppm	2.82d	4.2ab	4.48ab	4.58ab	4.02A	14.77ef	16.72bc	16.97b	18.52a	16.75A
300 ppm	2.53d	4.2ab	4.39ab	4.62a	3.94AB	15.22e	16.17cd	17.1b	18.97a	16.87A
Mean	2.72C	4.15B	4.30AB	4.47A		14.72D	16.05C	16.6B	18.61A	

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