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Full Length Research Paper

Improvement of plants salt tolerance by exogenous application of amino acids

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The present work has been performed to study the growth and metabolic activities of maize and broad bean plants which are shown to have a degree of sensitivity to salinity and to determine the role of amino acids proline or phenylalanine in increasing the salt tolerance of theses plants. Dry mass, water content, leaf area and photosynthetic pigment of maize and broad bean plants decreased with increasing salinity. These changes were accompanied with a drop in the contents of soluble sugars, soluble proteins and amino acids. This was accompanied by a marked increase in the proline content. When maize and broad bean plants sprayed with proline or phenylalanine the opposite effect was occurred, saccharides as well as proteins progressively increased at all sanitization levels and proline concentration significantly declined. Salinity significantly increased the sodium content in both shoots and roots of maize and broad bean plants, while a decline in the accumulation of K^+, Ca^{++}, Mg^{++} and P was observed. Amino acids treatments markedly altered the selectivity of Na^+, K^+, Ca^{++} and P in both maize and broad bean plants. Spraying with any of either proline or phenylalanine restricted Na^+ uptake and enhanced the uptake of K^+, K^+/Na^+ ratio, Ca^{++} and P selectivity in maize and broad bean plants.

Key words: Proline, phenylalanine, spraying, salinity, maize, broad bean.

INTRODUCTION

Soil solutions impose both ionic and osmotic stresses on plants. These can be distinguished at several levels. In salt-sensitive plants, shoot and to a lesser extent root growth is permanently reduced within hours of salt stress and this effect does not appear to depend on Na^+ concentrations in the growing tissues, but rather is response to the osmolarity of the external solution (Munns et al., 2000; Munns, 2002; Hamdia and Shaddad, 1997). Na^+-specific damage is associated with the accumulation of Na^+ in leaf tissues and results in necrosis of older leaves. Growth and yield reductions occur as a result of the shortening of the lifetime of individual leaves, thus reducing net productivity and crop yield (Munns, 1993, 2002). Osmotic damage (that is, osmotically driven removal of water evaporates could occur as a result of the build up of high concentrations of Na^+ in the leaf apoplast, since Na^+ enters leaves in the xylem stream and is left behind as water evaporates (Flowers et al., 1991; Katerji et al., 2000, 2001, Garacia et al., 2010). The cellular toxicity of Na^+ causes another type of osmotic problem.

Plants need to maintain internal water potential below that of the soil to maintain turgor and water uptake for growth. This requires an increase in osmotica, either by uptake of soil solutes or by synthesis of metabolically benign (compatible) solutes (Tester and Davenport, 2003). The stress-induced *Arabidopsis* and rice genes are thought to be involved in the plants response and tolerance to environmental stresses (Seki et al., 2002; Rabbani et al., 2003). Many plants accumulate compatible osmolytes, such as proline (Pro), glycine (Gly) betaine, or sugars, under osmotic stress. Pro biosynthesis from glutamine (Glu) appears to be the predominant pathway under stress conditions, because the repressed salt-stress ornithine omega-aminotransferase expression (the enzyme responsible for synthesis of Pro from Orn), induced the synthesis of Pro from Glu (Delauney and Verma, 1993; Delauney et al., 1993). Thus, the aim of the present work was to improvement the salt tolerance of maize and broad bean plants by exogenous application of proline or phenylalanine.

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MATERIALS AND METHODS

Maize (*Zea mays*) and broad bean (*Vicia faba*) plants were grown in plastic pots in the soil without NaCl (control) and under salinization levels corresponding to osmotic potential of NaCl solution of 0.3, 0.6, 0.9 and 1.2 MPa added to the soil in such a way that the soil solution acquired the assigned salinization levels at field capacity. Treatments of plants with saline solutions began when seedlings were two weeks old. The salinized and non-salinized plants were irrigated every other day with 1/10 Pfeffer's nutrient solution for two weeks. Then, proline or phenylalanine (100 ppm) solutions were added to the soil (5 intervals according to field capacity). The control plants were treated with distilled water. A week after the plants was used for analysis.

Dry matter was determined after drying plants in an aerated oven at 70°C to constant mass. Leaf area was measured by the disk method (Watson and Watson, 1953). Saccharides were determined by the anthrone-sulfuric acids method (Fales, 1951). Free amino acids, proline and a soluble protein contents were measured according to Moore and Stein (1948), Bates et al. (1973) and Lowry et al. (1951), respectively. Sodium and potassium were determined by flame-photometer method (Williams and Twine, 1960). Calcium and magnesium were measured by EDTA titration (Schwarzenbach and Biedermann, 1948) and phosphorus calorimetrically (Woods and Mellon, 1985).

RESULTS

Dry mass, water content, the leaf area and photosynthetic pigments regarded as a growth parameter decreased generally with the rise of salinization level according to the plant type (Tables 1 and 2). In broad bean plants, it remained unaffected up to -0.6 MPa, above which the values were reduced. In case of maize plants, salinity induced a sharp and progressive reduction in the values of leaf area especially at higher salinity level. Spraying the salinized plants with any of the two amino acids, proline or phenylalanine induced a significant increase in the total pigment contents and leaf area compared with untreated plants.

The soluble sugar contents in maize shoots and roots progressively decreased with the elevation of salinity levels (Table 3). In shoots of broad bean plants, the soluble sugar contents remained unchanged with increase of salinity levels, while in roots they decrease progressively. The soluble protein contents in shoots and roots of maize plants were appreciably lowered by salinity stress (Table 3). However, the pattern of this reduction was found to be constant at all salinity levels. In shoots it appeared mostly inconsistent. In shoot of broad bean plants, there was progressive decrease in nitrogen contents, in roots these content remained unaffected with rise of salinization levels. The amino acids content was decreased progressively in shoots and roots of maize and broad bean plants with increasing NaCl stress (Table 3). The addition of the experimental amino acids resulted sugar, soluble protein and amino acids in both shoots and roots of maize and broad bean plants as compared with control plants.

Proline concentration in shoots and roots of both maize and broad bean plants significantly increased with increasing salinity (Table 1). The accumulation of proline in root of maize plants was more than in roots while in broad bean plants, shoots accumulated more proline than in roots. Amino acids treatment markedly decreased proline accumulation in the shoots and roots of both plants (Table 1). Sodium concentration increased with the increased salinity (Table 4). The increase in Na^+ was much greater in broad bean plants than in maize plants. The distribution of Na^+ was much higher in roots than in shoots of maize plants, but was distributed evenly in shoots and in roots of broad bean plants. Salinity decreased the flux of sodium to the shoot of maize plant only. However, the flux of K^+ to the shoot of maize plants was more than in broad bean plants. Amino acids treatments retarded the absorption and consequently the accumulation of Na^+, especially in maize plants than in broad bean. Salinity decreased the accumulation of Ca^{++}, Mg^{++} and P of both maize and broad bean plants. Amino acids treatments significantly increase the accumulation of Ca^{++}, Mg^{++} and K^+/Na^+ ratio (Table 4).

DISCUSSION

Dry mass, water content, leaf area and photosynthetic pigment of maize and broad bean plants decreased with increasing salinity. These changes were accompanied with a drop in the contents of soluble sugars and soluble proteins. Osmotic and specific ion effects are the most frequently mentioned mechanisms by which saline substrates reduce plant growth. However, the relative importance of osmotic and specific ion effect on plant growth seems to vary depending on the salt tolerance of the plants. Such effects are resulted by decreasing the rate of water uptake due to osmo-effects, through ion-specific toxic effects, or through a nutritional imbalance as the result of inter-element antagonism (Levitt, 1980; Quayum et al., 1991; Hamdia and Shaddad, 1996; Najafi, 2010). Treatment with proline or phenylalanine (100 ppm) significantly increased these growth characteristics even at lowest salinity level tested.

Osmotic adjustment, defined as lowering of osmotic potential due to net solute accumulation in water stress, has been considered to be a beneficial drought tolerant mechanism for some crop species (Girma et al., 1992; Hamdia, 2002). When plants experience environmental stress, such as drought, high salinity, they activate various metabolic and defense system to survive. A number of genes corresponding to these stresses and their products were analyzed in *Arabidopsis* (Ono et al., 2003; Marayama et al., 2004) plants. For example, osmoprotectants, such as proline, glycine betaine, manitol and sugars confer stress tolerance (Yamada et al., 2005). The observed loss of soluble saccharides, soluble protein and amino acids of shoots and roots of salinized maize and broad bean plants was accompanied

Table 1. Effect of salinity stress and treatment with amino acids on dry matter (g plant $^{-1}$) on shoot and root of maize and broad bean plants grown for 45 days.

Maize	**NaCl**	**Dry weight**		**Content water**		**Proline**	
	-MPa	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
Control	0.0	0.31	0.41	8.2	4.7	0.07	0.08
	0.3	0.29	0.29	8.7	4.4	0.08	0.08
	0.6	0.24	0.24	5.3	3.6	0.9	1.2
	1.2	0.17	0.18	3.8	3.2	1.1	1.2
Proline	0.0	0.79	0.37	16.8	7.5	0.02	0.02
	0.3	0.28	0.36	8.5	6.4	0.03	0.02
	0.6	0.29	0.49	9.9	5.1	0.05	0.03
	1.2	0.39	0.44	6.7	3.8	0.06	0.04
Phenyl	0	0.52	0.53	12.2	6.3	0.01	0.01
	0.3	0.42	0.39	9.4	6.2	0.02	0.01
	0.6	0.37	0.39	9.4	.3.8	0.03	0.02
	1.2	0.25	0.15	6.9	5.3	0.03	0.03
L.S.D.%		0.175	0.02	1.8	0.76	0.02	0.03
Broad bean							
Control	0.0	1.19	1.18	7.3	3.1	0.09	0.04
	0.3	1.02	1.02	7	3.6	1.01	0.08
	0.6	0.94	0.94	5	2.9	1.2	1.0
	1.2	0.88	0.88	4.6	2.8	1.5	1.3
Proline	0	1.99	1.18	13.3	8.5	0.06	0.03
	0.3	2.48	1.02	14.9	6.9	0.08	0.02
	0.6	1.98	0.93	13.8	5.2	0.09	0.04
	1.2	1.57	0.66	6.9	6.2	1	0.03
Phenylalanine	0	1.63	1.36	9.9	6.7	0.04	0.03
	0.3	1.41	1.38	6.9	5.9	0.06	0.03
	0.6	1.67	1.77	7.7	8.3	0.06	0.04
	1.2	1.08	1.23	8.1	5.4	0.08	0.04
L.S.D. 5%		5.16	0.15	0.56	0.19	0.09	0.01

by a marked increase in the proline content. These results are in accordance with previously reported findings of Devitt et al. (1987), Hamdia and El-Komy (1998). It is worthy to mention that in our experiment when maize and broad bean plants where spraying with proline or phenylalanine the opposite effect was occured, saccharides as well as proteins progressively increased at all sainization levels. This was accompanied by an increased in amino acids of both shoot and root system. Thus, treatments with either proline or phenylalanine might play an important role in protein synthesis.

The physiological significance of proline accumulation is controversial, while some researches have reported that it is a sign of stress (Rai et al., 2003; Hernandez et al., 2000; Yamad et al., 2005). Other suggests that at a high concentration, it acts as solute intercellular osmotic adjustment (Silveria et al., 2003). Accordingly, their results dry matter production significantly decreased while proline accumulation was higher and detected earlier at a lower salinity concentration in broad bean plants compared to the maize plants, proline accumulation was higher in shoot than in root of broad bean plants, the opposite situation was observed between shoots and roots of maize plants.

Tissue water content remained unchanged in maize and broad bean at -0.3 MPa NaCl level, this was parallel with proline accumulation in both shoots and roots of maize plants. While proline accumulation significantly increases at -0.3 MPa in both shoots and roots of broad

Table 2. Effect of salinity stress and treatment with amino acids on pigment content (mg g^{-1} d.m.) and leaf area cm^2 of maize and broad bean plants grown for 45 days.

Maize	NaCl	Chl.a	Chl.b	Caroten.	Leaf area
Control	0.0	4.4	1.4	0.81	1.02
	0.3	4.8	1.6	0.91	71.7
	0.6	2.2	0.7	0.35	45.7
	1.2	1.2	0.65	0.22	40.8
Proline	0.0	4.9	1.0	1.2	139.9
	0.3	4.6	1.1	0.56	120.8
	0.6	4.5	1.3	0.63	65.8
	1.2	4.3	1.3	0.85	49.9
Phenlalanine	0.0	4.6	0.95	0.729	134.2
	0.3	4.0	1.1	0.89	143.8
	0.6	4.3	1.2	0.89	69.2
	1.2	4.5	1.0	0.71	60.3
L.S.D. 5%		1.3	0.34	0.08	4.3
Broad bean					
Control	0.0	1.5	0.4	0.49	24.9
	0.3	1.3	0.25	0.38	23.1
	0.6	1.2	0.29	0.34	20.3
	1.2	0.56	0.13	0.2	15.1
Proline	0.0	1.5	0.49	0.57	50.8
	0.3	1.7	0.55	0.41	51.8
	0.6	1.8	0.53	0.39	53.4
	1.2	1.4	0.34	0.47	47.9
Phenylalanine	0.0	1.5	0.58	0.41	30.5
	0.3	1.4	0.39	0.43	34.4
	0.6	1.7	39	0.47	33.3
	1.2	1.5	0.63	0.33	23.8
L.S.D. 5%		0.09	0.02	0.02	4.9

bean plants. It is worthy to point out that there are similar distribution of Na^+ in shoots and roots of broad bean plants, while the proline concentration was higher in shoots than in roots. The opposite situation was occurred in maize plants, where the concentration of both proline and Na^+ was higher in roots than in shoots. Our results show a lack of a consistent correlation between salinity tolerance and proline concentration.

When salt stressed maize and broad bean plant were sprayed with proline or phenylalanine, proline concentration significantly declined, while the amount of dry matter and water content for both maize and broad bean plants increased. This is accordance with the results obtained by (Shaddad and Heikal, 1982; Thakur and Rai, 1985; Hamdia 1987; Cuin and Shabala, 2005). This decline in proline concentration with salinity and treatment with amino acids was accompanied by a pronounced accumulation of other organic solutes (saccharides, protein and total amino acids). Treatment with proline or phenylalanine increased, to some extent, salt tolerance of these two plants through osmo-regulation, using the organic solutes rather than proline. This confirms the view of many authors (Manetas, 1990; Silveira et al., 2003; Yamada et al., 2005). The result here was in accordance with the result obtained by Cuin and Shabala (2005) has been suggested the role of compatible solutes in plant stress responses is not limited to conventional osmotic adjustment, but also includes some other regulatory or osmoprotective functions. One such function is in mainting cytosolic K^+ homeostasis by preventing NaCl induced K^+ leakage from the cell, a feature that may conifer salt tolerance in many species

Table 3. Effect of salinity stress and treatment with amino acids on soluble sugar (mg g^{-1} d.m.), total nitrogen (mg g^{-1} d.m.) and amino acids (mg g^{-}1 d.m.) of shoot and root of maize and broad bean plants grown for 45-days.

Maize	NaCl	Soluble sugar		Soluble protein		Amino acids	
		Shoot	Root	Shoot	Root	Shoot	Root
Control	0.0	44.9	67.3	39.2	31.2	38.7	24.1
	0.3	41.2	52.7	35.8	26.8	22.9	23.4
	0.6	37.6	39.6	32.7	26.5	20.8	14.2
	1.2	27.9	25.9	33.4	23.8	20.5	11.2
	0.0	60.3	73.3	41.5	52.2	56.6	35.8
Proline	0.3	57.7	70.9	37.5	34.6	54.5	32
	0.6	53.5	57.5	40.8	31.7	59.7	30.6
	1.2	33.4	39.6	35.6	26.9	45.5	24.2
Phenlalanine	0	52.9	69.4	37.5	44.3	72.7	29.4
	0.3	47.7	63.3	44.4	38.4	62.2	34.1
	0.6	83.2	58	44.4	36.8	47.5	27.5
	1.2	34.2	37.2	36.7	35.4	38.5	15.2
L.S.D. 5%		4.3	4.6	1.2	5.2	5.2	2.0
Broad bean							
Control	0.0	83	37.5	38.7	27.7	88.5	94.5
	0.3	74	39.6	40.7	26.3	87.1	63.1
	0.6	36.4	36.3	30.5	26.5	80.8	67.9
	1.2	34.3	36	25.9	26.6	72.4	45.2
Proline	0.0	72.4	57.1	45.6	42.6	96.4	115.2
	0.3	49.6	52.9	41.2	37.6	87.2	63.1
	0.6	54.7	60.6	36	35.5	80.8	60.5
	1.2	51.5	66.4	37.7	33.7	72.4	45.2
Phenylalanine	0.0	86.8	70.7	47.3	35.6	114	110.8
	0.3	72.4	66.9	46.4	40.8	77	61.5
	0.6	55.6	60.8	45.2	37.1	98.5	62.2
	1.2	49.2	63.8	43.4	32.5	105.6	43.9
L.S.D. 5%		5.8	14.5	2.3	3.3	3.3	5.6

particularly in barley (Botella et al., 1997; Cuin and Shabala, 2005). They showed also that low (-0.5 mM) concentrations of exogenously supplied proline significantly reduced NaCl induced K^+ efflux from barley roots in a dose-response manner. Also, Yamada et al. (2005) suggested that exogenous L-proline, plants treated with 5 mM L-proline accumulated up to 18 times more free than untreated plant. Hernandez et al. (2000) showed the capacity of tomato leaf tissues to accumulate proline in response to a salt shock (150 mM NaCl) applied to excised shoots, leafs, leaflets or leaf discs was determined and compared to that whole plants grown at the same salinity.

When plants experience environmental stresses, such as drought, high salinity, and low temperatures, they activate various metabolic and defense systems to survive. A number of genes corresponding to these stresses and their products were analyzed in *Arabidopsis* (Seki et al., 2002; Ono et al., 2003; Maruyama et al., 2004) and rice (Rabbani et al., 2003). Many genes and products commonly appear in response to drought, salinity, and low-temperature stresses. For example, osmoprotectants, such as proline (Pro), glycine betaine, manitol, and sugars confer stress tolerance. Transgenic plants have enhanced tolerance to drought and salinity and to drought and cold (Kavi et al., 1995; Huang et al., 2000; Abebe et al., 2003). Roosens et al., 1998 stated that in adult *Arabidopsis* plants, the free Pro increase was mainly due to the enzyme activity in the Glu pathway. However, in young *Arabidopsis* plants, the Orn and Glu

Table 4. Effect of salinity stress and treatment with amino acids on mineral content (mg g^{-1} d. m.) of shoot and root of maize and broad bean plants grown for 45 days.

Maize	NaCl	Shoot						Root					
		Na^+	K^+	K+/Na+	Ca++	Mg++	P	Na^+	K^+	K+/Na+	Ca++	Mg++	P
Control	0.0	4.3	14.7	3.4	1.9	1.2	1.5	8.8	8.1	0.92	2.0	1.3	1.6
	0.3	18.9	19.8	1.0	2.0	1.2	1.6	24..8	11.1	0.45	1.9	1.5	1.3
	0.6	30.1	15.3	0.51	1.8	1.2	1.2	35.3	13.8	0.39	1.2	1.2	1.2
	1.2	47.6	9.8	0.21	1.5	1.1	0.88	51.6	8.6	0.17	1.1	1.1	0.9
Proline	0	4.3	18.1	4.2	2.2	1.4	2.7	4.3	14.8	3.4	3.2	1.3	2.5
	0.3	13.1	22.5	1.7	2.4	1.4	3.1	13.1	19.2	1.5	2.7	1.5	2.3
	0.6	19.4	22.5	1.2	2.2	13	2.5	30.2	22.4	0.74	2.6	1.5	2.3
	1.2	25.8	26.2	1.0	2.2	1.2	2.1	45.2	31.7	0.7	2.6	1.5	2.1
Phenlalanine	0.0	4.6	11.4	2.5	2.1	1.6	2.1	2.3	10.9	4.7	2.0	1.8	2.8
	0.3	9.5	12.9	1.4	2.0	1.2	1.6	6.5	10.2	1.6	2.2	1.5	1.3
	0.6	16.8	15.8	0.94	2.1	1.1	1.5	13.4	15.9	1.2	2.1	1.1	1.1
	1.2	20.5	16	0.78	1.6	1.2	1.1	25.8	25.8	1.0	1.9	1.2	0.97
L.S.D. 5%		0.77	1.4	1.0	3.0	0.8	0.04	0.144	0.189	0.44	5.1	0.59	0.15
Broad bean													
Control	0.0	15.1	12.7	0.84	2.0	7.3	1.0	10.5	7.7	0.73	2.3	1.5	1.0
	0.3	30.5	10.8	0.35	2.3	5.1	1.0	38.2	9.2	0.24	2.5	1.5	0.8
	0.6	45.1	10.8	0.24	2.0	5	0.85	45.3	10.2	0.23	1.9	1.1	0.7
	1.2	57.3	10.2	0.18	1.5	4.9	0.63	67.7	11.9	0.18	1.4	1.1	0.3
Proline	0.0	10.3	8.7	0.84	2.3	1.3	2.1	7.5	7.1	0.95	2.7	1.5	1.9
	0.3	17.5	9.3	0.53	1.6	1.1	2.2	38.7	9.3	0.24	2.3	1.3	1.9
	0.6	25.1	8.9	0.35	1.4	1.3	2	42.4	7.5	0.17	2.1	1.4	1.3
	1.2	45.2	9.1	0.2	1.2	1.5	1.7	65.1	6.8	0.1	2.1	1.4	1.2
Phenylalanine	0.0	13.8	10.3	0.75	1.8	1.0	1.9	7.5	17.1	2.3	1.9	1.9	1.9
	0.3	32.3	9.4	0.29	2.5	7.8	1.5	41.9	14.0	0.33	1.6	1.8	1.0
	0.6	35.2	10.2	0.29	2.2	7.0	1.7	48.6	13.0	0.27	1.2	1.2	1.4
	1.2	48.4	9.6	0.19	1.3	8.0	1.1	54.5	10.7	0.19	1.1	1.1	0.7
L.S.D. 5%		8.6	0.67	1.2	2.2	1.2	0.09	10.8	2.0	0.54	3.0	1.6	0.06

pathways together play an important role in accumulating Pro during osmotic stress. It is suggested that P5CS is an important enzyme for Pro biosynthesis and accumulations in young petunia plants. It also increases their ability to withstand drought and salinity stresses.

Crop performance may adversely affected by salinity-induced nutritional disorders. These disorders may result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within plant (Grattan, 1999; Katerji et al., 2000, 2001). In their results, salinity significantly increase in leaf area and photosynthetic pigment was concomitant by increase in the Mg^{++} content leading to the more accumulation of saccharides as a result of spraying with each amino acids 100 ppm proline or phenylalanine.

Amino acids treatments markedly altered the selectivity of Na^+, K^+, Ca^{++} and P in both maize and broad bean plants. Spraying with any of either proline or phenylalanine restricted Na^+ uptake and enhanced the uptake of K^+, K^+/Na^+ ratio, Ca^{++} and P selectivity in maize and broad bean plants. However, maize plants have a lower Na^+ in shoot and root, concomitant with a marked decrease in growth (dry matter and water content) as a result of amino acids treatment compared to broad bean while an increase Na^+ content was observed with a marked increase in growth criteria. Thus, besides the possible effect of amino acids treatment in reducing Na^+ concentration in maize tissue, an increase in water content may be responsible for increased growth in salinity and amino acids treatments (Cramer et al., 1985;

Hamdia et al., 2004). Generally, it can be said that the exogenous amino acids treatments might counteract the negative effects of salinity exerted on saccharides, nitrogen metabolism and mineral, which consequently could promote the plant growth.

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