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ORIGINAL ARTICLE

The Response Strategy of Maize, Pea and Broad Bean Plants to Different Osmotic Potential Stress

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This investigation was conducted to study the tolerance strategy of maize, broad bean and pea plants to salinity stress with exogenous applications of proline or phenylalanine on seed germination and seedlings growth. From the results obtained, it can be observed that osmotic stress affected adversely the rate of germination in maize, broad bean and pea plants. The excessive inhibition was more prominent at higher concentration of NaCl. The seeds and grains tested were exhibited some differential responses to salinity, in a manner that the inhibitory effect of salinity on seed germination ran in the order, maize higher than broad bean and the later was higher than pea plant. Treatment with proline or phenylalanine (100 ppm) significantly increased these seed germination and seedlings growth characteristics even at lowest salinity level tested.

Key words: Salinity, broad bean, maize, pea, proline, phenylalanine

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Key words: Salinity, broad bean, maize, pea, proline, phenylalanine

Abrevation: Proline: Pr, phenylalanine: Phe

Abiotic stress tolerance is a complex trait that involves tolerances to salinity, extreme temperatures, soil drought, or limited mineral nutrients. The combinations and severity of these stresses that crops must tolerate vary with environment. Salinity is a major environmental factor that limits crop growth and productivity (Hamdia and Shaddad, 2010). It is estimated that 20 % of all cultivated land, and almost half of all irrigated land, is affected by salinity (Rhoades and Loveday, 1990; Kabala and Russak, 2012). To cope with salt stress, plants have developed multifarious adaptation mechanisms to tolerate high concentrations of salt in the environment (Hamada *et al.*, 2001). Amino acids help plants to resist stresses and defend against pests and diseases (Wallsgrove, 1995). Plants convert inorganic nitrogen into amino acids, the building blocks of proteins, and a variety of other functional compounds. However, when plants are under stressful conditions, they are unable to perform their normal physiological activities to synthesize their own amino acids. The exogenous application of L- amino acids reduces the energy requirements of plants and this saved energy can be used for vital processes, especially under extreme adverse conditions Watschke and Borger (2012) wark on *Turfgrass* under heat stress treated with amino acid biofertilizers had better quality than the control.

Many studies have demonstrated that exogenous supplied osmoprotectants are able to improve plant tolerance under abiotic stress. In particular Kadota *et al*. (2001) demonstrated that Japanese pear that suppling sorbitol to growth media improved the shoot proliferation efficiency and fresh mass. Supplementing sorbitol in the growth media dramatically increased plantlets regeneration frequency from derived calli of four rice cultivars (Geng *et al*., 2008; Teerakulpisut and Gunnula, 2012; Rajjou *et al*., 2012; Liu *et al*., 2012)

Amino acids are key factors in metabolism and development of higher plants. Moreover, amino acids act as signaling molecules, controlling their own metabolism and the expression of a variety of genes (Kiyosue *et al*., 1996; Nielsen *et al*., 1998). Under environmental stress conditions such as salt, cold, and drought stress, many plants accumulate compatible solutes such as Pro and Gly betaine. The function of Pro under stress conditions is not fully understood. The amino acid is discussed as a compatible osmolyte, which, in addition, might serve as a protectant of macromolecules or even as a scavenger of hydroxyl radicals (Smirnoff and Cumbes, 1989). Furthermore, Pro can serve as a rapidly available source of nitrogen, carbon, and reduction equivalents during recovery from stress (Ahmad and Hellebust, 1988; Hellman *et al*., 2000; Hamdia *et al*., 2012)

Thus the present investigation was conducted to strategy behavior of maize, pea and broad bean plants to salt stress and with exogenous application of proline and phenylalanine at germinating stages.

MATERIALS AND METHODS

The present work was carried out to study the effect of salinization levels on three of economic plants Maize (*Zea mays*), pea (*Pisum sativum*) and broad bean (*Vicia faba*) in addition the role of amino acids (proline or phenylalanine) in alleviation the effect of salinization levels on the germination percentage, growth parameters at seedling stage (length, fresh and dry matter of shoot and root).

Determination of germination percentage:

The germination experiments were performed as described by Maftoun and Sepaskhah (1973). The following osmotic potentials -0.3, -0.6, -0.9 and -1.2 MPa NaCl levels were used in addition of control. The seeds of three tested plants were sterilized by ethanol and H_2O_2 with ratio 1:1 volume for 3 minutes, the seeds were further washed with distilled water and placed on absorbent pads

In petri dishes placed 30 ml of the previous experimental solution were added. Twenty seeds were considered to be germinated after radical emerged from the test. To evaluate the interactive effect of both proline and phenylalanine (100 ppm) and osmotic stress on seed germination, each of the two amino acids was added to the culture medium according to the field capacity. The percentage germination of seeds was followed daily for a period of 3 to 4 days. After 10 days of germination the length of radical and hypocotyle of germinated seeds were measured.

Fresh and dry matter of germinated seeds

At the end of the experimental period the fresh matter of the seedlings were dried in aerated – oven at 70 ^{0} C until constant dry matter reached.

Statistical analysis:

The data of all experiments were subjected to one way analysis variance and means were compared using the least significant difference test (L.S.D.) using statistical program (Sta. Base. Exe.) on computer (Steel and Torrie, 1960).

RESULTS

Maize

Germination percentage of maize seeds did not exhibited any significant changes in germination ability up to -0.6 MPa, after that a marked decrease in germination percentage was observed at all days of germination as compared with control plants (0.0) (table 1). The values of reduction in the germination percentage at- 0.9 and -1.2 NaCl levels was 19% and 31% as compared with control plants (100%) at 4th day of germination respectively. The fresh and dry matter, water content and the length of radical and hypocotyle of variously salinized maize seedlings were reduced progressively with the increase of salinization levels up to -0.6 MPa NaCl (tables 2, 3, 4). The percent of reduction in fresh and dry matter at 0.6 MPa NaCl levels was 33% and 45% respectively. It can be observe that, while the maize seeds can germinated at higher levels of salinity (-0.9 MPa and -1.2 MPa), it can not complete the seedling growth stages.

Exogenously application of Pro or Phe resulted in a significant increase in the percent maize grain germination, length of radical and hypocotyle and fresh and dry matter as compared with control plants (table 1). The increase in the percent of maize germination at -0.9 MPa and -1.2 MPa salinization levels was 93% and 89.1% respectively for proline treatments. In case of grain treated with Phe this activation was 89.1% and 88% at -0.9 MPa and -1.2 MPa NaCl levels respectively. The interesting result was that maize seeds complete the seedling growth stages with treatments with either Pro or Phe. Proline treatments increase the percent of fresh and dry matter production at -0.6 MPa 38% and 49% and for Phe treatments was 77% and 73% for fresh and dry matter respectively. The percent increase of radical and hypocotyle length at -0.6 MPa was 31% and 107 for proline treatment and 41% and 83% for Phe treatment (tables 2, 4).

Pea

The percent of pea germination represented in table 2 was markedly reduced with the increase of osmotic stress levels at all days of germination up to -0.6MPa. Moreover the higher salinization levels used (-0.9 and -1.2 MPa) used completely inhibited the germination of pea seeds (table 5). The reduction in the percent germination at -0.6 MPa was 42.9% as compared with untreated plants at 4th day of germination. The production of fresh and dry matter, water content as well as shoot and root lengths of variously salinized pea seedlings were significantly reduced with rise of salinization levels up to -0.6 MPa NaCl, after that these parameters were greatly inhibited as compared with untreated seeds (tables 2, 3, 4). Exogenous treatments with either Pro or Phe were enhanced the percent of germination of pea seeds whatever the salinization level used. The percent of seed germination at -0.6 MPa was 98% in seed treated with proline and 70% in seeds treated with Phe (table 5). Treatments with either Pr or Phe of variously salinized pea seedlings induced a significant increase in the values of seedlings growth parameters (fresh and dry matter, water content and radical and hypocotyle length) (tables 2, 3, 4).

Broad bean

The percent of broad bean seed germination smoothly decreased with the increase of osmotic stress up to the level -0.6 MPa, after that at the highest NaCl levels -0.9 and -1.2 MPa used, the germination percentage was completely inhibited at all days of germination (table 5). The reduction in the value of germination percentage at -0.9 MPa was 10% as compared with unsalinized plants (table 6). Salinity induced a considerable reduction in the values of fresh and dry matters, water content and radical and hypocotyl lengths of broad bean seedlings as compared with those of non-salinized seedlings (tables 2, 3, 4). Amino acids treatments (Pro or Phe) activated the germination of salinized broad bean plants. This stimulatory effect was more significantly at relatively higher salinity levels. The percent of seed germination at -0.6 MPa was 100% in case of seeds treated with Pro and 98% and in case of seeds treated with Phe. The exogenously addition of amino acids (Pro or Phe) to the variously salinized broad bean seedlings significantly activated fresh and dry matter, water content and radical and hypocotyl lengthes as compared with control plant (tables 2, 3, 4).

Table 1. Effect of different osmotic stress and treatments with aminoacids proline or phenylalanine on germination percentage of seedling of maize plants at the 10 days from planting.

	NaCl -MPa	**1st day**	**2nd day %**	**3rd day %**	**4th day %**
Control	0.0	0.0	76.7 100	98.3 100	98.3 100
	0.3	0.0	76.7 100	96.7 98	100 100
	0.6	0.0	70 91	89.3 90	96.7 98
	0.9	0.0	41 41	71.7 72	80 81
	1.2	0.0	30 30	60.0 61	86.3 69
Proline	0.0	0.0	88.3 115	98.3 100	98.3 100
	0.3	0.0	86.3 113	95.0 96	96.7 98
	0.6	0.0	68.3 89	88.3 89	96.7 98
	0.9	0.0	46.7 60	88.3 89	91.7 93
	1.2	0.0	41.7 54	70.0 71	87.7 89
Phenyl-alanine	0.0	0.0	88.3 115	98.3 100	98.3 100
	0.3	0.0	80.3 104	90.0 91	98.3 100
	0.6	0.0	78.3 102	91.7 93	93.3 100
	0.9	0.0	50.0 65	81.7 83	88.3 89
	1.2	0.0	41.7 54	78.3 79	86.7 88
L.S.D. 5%			2.2	3.2	3.5

Table 2. Effect of different osmotic stress and treatments with amino acids proline or phenylalanine on fresh and dry matter (g seedling^{-1}) of seedling of maize, pea and broad bean plants at the 10 days from planting.

	NaCl -MPa	Maize				Pea				Broad bean			
		f.m.	%	d.m.	%	f.m.	%	d.m.	%	f.m.	%	d.m.	%
Control	0.0	3.12	100	0.39	100	0.40	100	0.05	100	3.5	100	0.35	100
	0.3	1.35	43	0.19	49	0.31	77	0.04	82	2.3	65	0.29	81
	0.6	1.04	33	0.18	45	0.31	77	0.04	80	0.96	27	0.10	29
	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0..0	0.0	0.0	0.0	0.0
Proline	0.0	5.75	148	0.60	152	0.44	107	0.07	145	3.35	95	0.33	93
	0.3	3.22	103	0.23	75	0.39	97	0.07	133	2.3	65	0.29	82
	0.6	1.01	38	0.16	49	0.26	64	0.06	109	1.5	43	0.15	43
	0.9	0.32	10	0.05	11	0.05	12	0.003	5.9	0.47	13	0.08	22
	1.2	0.09	2.9	0.01	1.7	0.03	7.9	0.001	1.9	0.04	1.2	0.01	3.7
Phenylalanine	0.0	4.17	133	0.57	143	0.49	121	0.05	98	3.8	107	0.41	117
	0.3	3.9	125	0.37	91	0.45	111	0.04	82	2.4	67	0.29	82
	0.6	2.43	77	0.29	73	0.39	98	0.03	66	1.4	39	0.17	48
	0.9	1.03	33	0.12	31	0.07	17	0.004	7.8	0.20	5.7	0.05	13
	1.2	0.05	1.7	0.03	8.0	0.04	10	0.002	3.9	0.05	1.3	0.02	5.7
L.S.D. 5%		0.21		0.193		0.350		0.015		0.193		0.011	

Table 3. Effect of different osmotic stress and treatments with amino acids proline or phenylalanine on water content of maize, pea and broad bean plants at the 10 days from planting.

	NaCl - MPa	**Maize**		**Pea**		**Broad bean**	
		W.C.	%	W.C.	%	W.C.	%
Control	0.0	2.7	100	0.35	100	3.15	100
	0.3	1.1	43	0.27	77	2.0	63
	0.6	0.86	31	0.27	77	0.86	27
	0.9	0.0	0.0	0.0	0.0	0.0	0.0
	1.2	0.0	0.0	0.0	0.0	0.0	0.0
Proline	0.0	5.2	192	0.37	105	3.02	95
	0.3	3.5	107	0.32	91	2.01	63
	0.6	2.1	31	0.2	57	1.35	43
	0.9	0.91	6.3	0.05	14	0.39	12
	1.2	0.02	2.9	0.03	8.5	0.03	0.9
Phenylalanine	0.0	3.6	133	0.44	125	3.4	107
	0.3	3. 5	129	0.41	117	2.1	66
	0.6	2.1	77	0.36	102	1.2	38
	0.9	0.91	33	0.07	20	0.15	47
	1.2	0.02	0.7	0.04	11	0.03	0.95
L.S.D. 5%		0.02		0.03		0.01	

Table 4. Effect of different osmotic stress and treatments with amino acids proline or phenylalanine on length of shoot and root (Cm) of seedling of maize, pea and broad bean plants at the 10 days from planting.

	NaCl -MPa	Maize		Pea		Broad bean	
		Radical %	Hypo. %	Radical %	Hypo. %	Radical %	Hypo. %
Control	0.0	9.4 100	5.1 100	2.5 100	0.58 100	1.7 100	0.80 100
	0.3	3.3 35	4.6 89	1.3 51	0.23 40	1.0 63	0.34 42
	0.6	2.2 23	3.0 60	0.78 30	0.23 39	0.5 29	0.20 52
	0.9	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
	1.2	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Proline	0.0	11 124	4.9 95	2.5 96	0.58 100	1.8 102	0.90 113
	0.3	5.1 53	5.1 99	1.9 76	0.35 60	1.0 59	0.51 103
	0.6	3.0 31	5.5 107	1.6 63	0.31 54	0.53 31	0.20 25
	0.9	2.2 23	4.5 87	0.82 32	0.12 21	0.3 17	0.09 11
	1.2	0.99 10	0.50 9.7	0.52 20	0.09 15	0.09 5.2	0.05 6.3
Phenylalanine	0.0	9.9 104	5.6 108	2.5 98	0.72 125	1.8 104	0.98 112
	0.3	4.8 50	5.7 110	1.8 71	0.29 50	1.0 58	0.43 53
	0.6	3.9 41	4.3 83	1.3 51	0.28 48	0.64 37	0.35 43
	0.9	1.3 13	3.3 64	0.8 31	0.12 20	0.35 37	0.20 25
	1.2	0.9 9.5	0.25 4.7	0.2 7.9	0.01 1.7	0.09 20	0.07 8.8
L.S.D. 5%		1.4	1.6	0.23	0.01	0.06	0.03

Table 5. Effect of different osmotic stress and treatments with amino acids proline or phenylalanine on germination percentage of seedling of pea plants.

	NaCl-MPa	1st day	2nd day %	3rd day %	4th day %
Control	0.0	0.0	73.3 100	98.3 100	100 100
	0.3	0.0	63.3 86	67.7 68	80.0 80
	0.6	0.0	15.0 20	50.0 50	58.3 58
	0.9	0.0	0.0 0.0	0.0 0.0	0.0 0.0
	1.2	0.0	0.0 0.0	0.0 0.0	0.0 0.0
Proline	0.0	0.0	83.3 113	100 101	100 100
	0.3	0.0	91.7 125	86.7 88	100 100
	0.6	0.0	70.0 95	98.3 100	98.3 98
	0.9	0.0	16.7 22	23.3 23	25.0 25
	1.2	0.0	0.33 0.4	5.0 5.1	10.0 10
Phenylalanine	0.0	0.0	71.7 96	69.7 70	100 100
	0.3	0.0	40.0 54	60.0 61	90.0 98
	0.6	0.0	36.7 50	60.0 61	70.0 70
	0.9	0.0	11.7 50	16.7 16	16.7 16.7
	1.2	0.0	6.7 15.9	9.7 9.8	11.7 11.7
L.S.D. 5%		0.0	3.2	2.5	3.2

Table 6. Effect of different osmotic stress and treatments with amino acids proline or phenylalanine on germination percentage of seedling of broad bean plants.

	NaCl-MPa	1st day	2nd day %	3rd day %	4th day %
Control	0.0	0.0	34.3 100	100 100	100 100
	0.3	0.0	31.7 92	87.3 87.3	93.3 93.3
	0.6	0.0	28.3 82	68.3 68.3	93.3 93.3
	0.9	0.0	0.0 0.0	0.0 0.0	10 10
	1.2	0.0	0.0 0.0	0.0 0.0	0.0 0.0
Proline	0.0	0.0	60.0 174	98.3 98	100 100
	0.3	0.0	40.0 116	68.7 68	100 100
	0.6	0.0	35.0 102	78.3 78	100 100
	0.9	0.0	0.0 0.0	6.7 6.7	55 55
	1.2	0.0	0.0 0.0	0.0 0.0	6.7 6.7
Phenylalanine	0.0	0.0	55.0 160	100 100	100 100
	0.3	0.0	31.7 92	80.0 80	100 100
	0.6	0.0	33.3 33.3	67.7 67.7	98.0 98
	0.9	0.0	0.0 0.0	0.0 0.0	15.0 15
	1.2	0.0	0.0 0.0	0.0 0.0	8.3 8.3
L.S.D. 5%	0.0	0.0	2.4	2.5	3.3

DISCUSSION

This investigation was conducted to study the tolerance strategy of maize, broad bean and pea plants to salinity stress with exogenous applications of proline or phenylalanine on seed germination and seedlings growth. From the results obtained, it can be detected that osmotic stress affected adversely the rate of germination in maize, broad bean and pea plants. The excessive inhibition was more prominent at higher concentration of NaCl. The seeds and grains tested were exhibited some differential responses to salinity at seed germination period, in a manner that the tolerance to the inhibitory effect of salinity on seed germination run in the order, maize higher than broad bean and the later was higher than pea. It seems that this reduction in seed germination under salinization treatments is as the result of osmotic and specific ion effects the most frequently mentioned mechanisms by which saline substrates reduce seedlings growth. However, the relative importance of osmotic and specific ion effect on seedling growth seems to vary depending on the salt tolerance of the plants. NaCl had an inhibitory effects on the water uptake of maize, pea and broad bean plants. Water generally plays the most important role in the process of seed germination, but high salinity prevents the seeds from absorbing enough water. Such effects are resulted by decreasing the rate of water uptake due to osmo-effects, through ions 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 and Hamdia *et al.,* 2012). A broader application of the concept is to apply low- or high-salinity water in synchrony with salt-sensitive or salt-tolerant growth stages of a single crop. Such a strategy would extend the use of both low- and high-quality water. Drainage water cannot be discharged and this is one way it could be used to decrease its volume.

From the obtained data, it can be seen that, while maize grains can germinated especially at higher salinization levels (-0.9 MPa and -1.2 MPa) (table 1), they can not survive and complete the seedling growth. The maize seeds in that situation were different from pea and broad bean seedlings which they can not germinated and complete the seedling growth period at these levels of salinity (-0.9 MPa and -1.2 MPa). The data of fresh, dry matter and water content of three tested plants at 0.0, -0.3 MPa and -0.6 MPa were explain that pea seeds preserve water content and product higher fresh and dry matter than broad bean and maize seeds. This means that maize plants can tolerated the deleterious effect of salinity than broad bean and pea plants at germinating stage while at seedling growth stages the data of seedling growth parameters (fresh and dry matter of radical and hypocotyle as well as the water content) explain that pea plants can tolerated and increase these values than broad bean and maize plants. This can be throw the light that plants differ in their strategy tolerance not between different plants at different stages but the same plant different its response at the same stage (at germination days and seedling growth). Many plants are most sensitive to ion stress during germination (Catalan *et al.*, 1994; Katembe *et al*., 2012) or young seedling growth (Rogers *et al*., 1995; Carvajal *et al*., 1998). Other researchers have also demonstrated that plants exhibit different sensitivities to salinity at different stages of growth (Maas and Poss, 1989; Francois, 1994). More recently, Steppuhn and Wall (1997) showed that, in the case of wheat, adding salts after emergence resulted in greater salt tolerance than subjecting plants to full salinity at seeding. However, the exact physiological mechanisms involved in these observations remain to be elucidated (Wilson *et al*., 2000).

Treatment with proline or phenylalanine (100 ppm) significantly increased these seed germination and seedlings growth characteristics even at lowest salinity level tested (Hamdia and Shaddad, 2010; Hamdia *et al*., 2011; Katembe *et al*., 2012). Timoshchenko *et al.* (2000) study the initial stage of amino acid metabolism was intensified in germinating wheat seeds with exogenous glutamine and proline. Exogenous glutamine and proline accumulated over 2 h at 4~ in swelling seeds were spent at different rates over the following 2 h at 20~ thus compensating for insufficiency of these amino acids during the initial stage of development. Creation of additional pools of glutamine and proline during the initial stage of amino acid metabolism had positive effects on the seed germination and vital activity of the plants. Song *et al*. (2012) showed that the seed germination percentage decreased with increasing NaCl concentration, and at the high NaCl level (800 mM), the lowest germination percentage was recorded. At the low NaCl levels, the highest germination rate was observed on day two and the seedling length was promoted slightly. In contrast, the germination delayed and the seedling length decreased at the high salinity

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