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Alleviation of Salinity Stress on Growth and Some Physiological Activities of Kidney Bean Using Proline or Phenylalanine

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ABSTRACT. The objective of this study was to investigate the effects of various concentrations of NaCl (0, 1, 3, 5 bars) as a salinizing agent on growth, pigment content and concentration of some minerals in kidney bean plants grown in sand culture. Also, particular interest was focused on effects of treatment on the stressed plants sprayed with any of the two amino acids (proline and phenylalanine) to test whether these amino acids can alleviate, or at least modify, the pattern of changes induced by the salinization treatments.

Generally, a significant reduction in all parameters studied of kidney bean was induced by salinization. Spraying with any of the two amino acids was generally effective in ameliorating the adverse effect of salinity on growth, pigment content and concentration of nutritive elements K, Ca, Mg, P and N, especially at the relatively moderate and high salinity levels (3 and 5 bars).

Introduction

The salt tolerance of plants and the changes in their physiological activities and growth have received considerable attention by some authors[1-6]. In this respect, other authors recorded a considerable accumulation of free proline in plants exposed to water stress or salinity[7-9]. Also Singh *et al.*[10] mentioned that resistant varieties of barley exposed to osmotic stress accumulated more proline than non-resistant ones and addition of oxogenous proline helped the non-resistant varieties to overcome the

stress. In addition, Heikal and Shaddad[11], Shaddad and Heikal[12], and Thakur and Rai[13] reported that exogenous applications of proline reversed the negative effects of stress conditions in some glycophytes.

In view of these findings, it was of interest to study the effects of some exogenously added amino acids on some salt-sensitive plants. Therefore, the aim of the present study was to evaluate the interactive effects of salt stress and the amino acids, proline and phenylalanine on growth, pigment content and minerals content of kidney bean plant.

Material and Methods

Seeds of kidney beans (*Phaseolus vulgaris* var. *strike*) were used. The seeds were sown in perforated plastic pots, each containing two kgs of soil, composed of mixed sieved, acid washed sand and peat moss (3:1 by volume). The pots were irrigated with tap water till complete emergence. The pots were divided into four groups of five pots each, then watered with ½ strength Hoagland nutrient solution containing various concentrations of NaCl equivalent to the stress levels 0,1,3, and 5 bars. Every two days, each group was irrigated with the respective saline solution. Two seedlings per pot were left to grow in green house at about 25°C at a soil water potential near field capacity. In order to prevent accumulation of salts, the soil in each pot was leached every 10 days with excessive amount of tap water.

Another experiment was carried out simultaneously to evaluate the effect of the amino acid proline or phenylalanine and sodium chloride on kidney bean plant. In this experiment another group of kidney bean plants was treated with different saline solutions as mentioned above. In addition, hence were sprayed with 100 ppm proline or phenylalanine. Two spray applications were carried out, the first (10 ml for each pot) after one week and the second (15 ml for each pot) after two weeks of salinization. The spray applied with a small atomizer, completely covered all the leaves, but did not drip.

At the end of the experimental period (5 weeks), the photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined using spectrophotometric method recommended by Metzner *et al.*[14]. Also, shoots and roots of treated plants were harvested and weighed. They were oven-dried at 70°C to constant weight.

The dry samples were ground into fine powder and assayed for minerals determination using the wet digestion method[15]. The flame photometer absorption method was used for Na, K, Ca and Mg determinations using atomic absorption flame photometer of the type Shimadzu AA-670/G U-A. Phosphorus was determined colorimetrically using the phosphomolybdate method[16]. Total nitrogen was also determined colorimetrically by the method adopted by Delory[17] using Nessler reagent.

In every case, at least, three replicates were used and the data were statistically analysed to calculate the least significant difference.

Results

Fresh and Dry Weights

Fresh and dry weights of kidney bean as influenced by salinization levels and amino acid treatments are given in Table 1. The results showed that fresh and dry weights of shoot and root systems, as well as of the whole plant, were reduced with the rise of salinization level. This reduction was more pronounced at relatively moderate and high salinity levels (3 and 5 bars).

TABLE 1. Effect of salinization levels and proline or phenylalanine treatments on fresh and dry weight yields (g/plant) of kidney bean.

Amino acid treatments	Salinization level (bars)	Fresh weight			Dry weight		
		Shoot	Root	Whole plant	Shoot	Root	Whole plant
Reference control	0	12.15	2.20	14.35	1.58	0.20	1.78
	1	11.43	2.25	13.68	1.45	0.19	1.64
	3	8.22	1.53	9.75	1.05	0.15	1.20
	5	6.70	1.30	8.00	0.85	0.11	0.96
Proline	0	13.74*	2.35	16.09*	1.69*	0.21	1.90*
	1	12.84*	2.34	15.18*	1.67*	0.21	1.88*
	3	10.98*	2.08*	13.06*	1.37*	0.18*	1.55*
	5	9.37*	1.60	10.97*	1.21*	0.15*	1.36*
Phenylalanine	0	13.04*	2.24	15.28*	1.65*	0.20	1.85
	1	11.73	2.31	14.04	1.57*	0.20	1.77*
	3	10.34*	1.74	12.08*	1.26*	0.16	1.42*
	5	8.67*	1.43	10.10*	1.05*	0.13	1.18*
L.S.D. 5%		0.44	0.35	0.63	0.07	0.03	0.11

*Significant differences as compared to the reference control plants.

Generally, spraying with proline or phenylalanine resulted in a significant increase in the values of fresh and dry weights of shoots and roots as well as of the whole plant at all salinization levels as compared to those of reference control plants. The increase in fresh and dry weight of roots of plants sprayed with phenylalanine was non-significant at all salinity levels. It can be noticed that, spraying with proline solution was more effective in increasing growth than spraying with phenylalanine solution at all salinization levels. Furthermore, it can be noticed that plants grown at the lowest level of salinization (one bar) and treated with proline exhibited better growth compared to the reference control plants (0.0 NaCl).

Photosynthetic Pigments

From the data given in Table 2, it can be seen that the content of pigment fractions and consequently of the total pigment content of salinized kidney bean plants were significantly decreased with the rise of salinization level. This decrease was more pronounced at relatively moderate and higher levels of sodium chloride (3 and 5 bars).

TABLE 2. Effect of salinization levels and proline or phenylalanine treatments on pigment content (mg/g dry weight) of kidney bean leaves.

Amino acid treatments	salinization level (bars)	Chl. a	Chl. b	Carot.	Total pigments
Reference control	0	11.32	7.93	5.16	24.41
	1	9.83	6.69	3.72	20.24
	3	7.56	5.23	3.84	16.63
	5	7.20	4.58	3.65	15.43
Proline	0	11.63	8.45	6.06*	26.14
	1	9.92	7.37	5.06*	22.35
	3	9.50*	7.03*	5.15*	21.68*
	5	9.40*	6.41*	4.04	19.85*
Phenylalanine	0	12.08	7.12	5.86*	25.06
	1	10.12	6.37	5.13*	21.62
	3	9.67*	4.96	4.88*	19.51*
	5	8.68*	5.20	4.08	17.96*
L.S.D. 5%		0.93	0.90	0.62	2.15

*Significant differences compared to the reference control plants.

Spraying with any of the two amino acids (proline or phenylalanine) induced a stimulatory effect on the content of pigment fractions as well as total pigments. This stimulatory effect was more pronounced in case of total pigment content of plants sprayed with proline than those sprayed with phenylalanine. It can also be noticed that the change in chlorophyll b content in plants sprayed with phenylalanine was nonsignificant compared to the reference control plants.

Mineral Composition

The influence of various salinization levels and amino acid treatments on the contents of some elements in the tissues of kidney bean plant are presented in tables 3 and 4.

Sodium content of shoots and roots of kidney bean increased significantly and progressively with the rise of salinity level. However, its accumulation was comparatively less in shoots than in roots (Tables 3 and 4). Contrary to sodium, the concentrations of the other nutritive elements tested (K, Ca, Mg, P, N) generally decreased in both shoots and roots with the rise of salinity level.

Spraying with either proline or phenylalanine prohibited the accumulation of sodium, regardless of the salinization level or the plant organ analysed. The magnitude of this reduction was relatively higher in plants sprayed with proline, in comparison with those subjected only to corresponding levels of salinization (reference control). Spraying with any of the two amino acids generally induced a significant increase in the concentrations of most of the nutritive elements tested in both shoots and roots. This was especially true at the relatively moderate and higher levels of NaCl (3 and 5 bars) compared to the control plants. This stimulatory effect was more pronounced in plants sprayed with proline than in those sprayed with phenylalanine.

TABLE 3. Effect of salinization levels and proline or phenylalanine treatments on the content of some nutritive elements in kidney bean shoots. Data are expressed as mg/g dry weight.

Amino acid treatments	Salinization level (bars)	Na	K	Ca	Mg	P	N
Reference control	0	1.23	26.73	28.65	15.33	5.47	39.56
	1	5.17	25.38	25.64	14.59	4.90	38.97
	3	15.73	22.46	22.18	13.00	4.51	37.03
	5	24.59	19.87	21.05	12.29	4.01	32.63
Proline	0	1.01	28.23*	28.29	15.80	5.52	40.09
	1	3.95*	26.87*	26.30	15.95*	5.20	41.11
	3	12.07*	24.75*	26.00*	15.19*	6.61*	41.04*
	5	19.22*	23.33*	25.28*	14.49*	6.05*	36.36*
Phenylalanine	0	1.11	27.56*	29.17	16.00	5.97*	40.82
	1	4.07*	26.14*	25.93	15.97*	5.60*	41.92*
	3	12.84*	24.43*	25.63*	14.74*	5.79*	39.30
	5	19.91*	23.09*	24.81*	13.90*	5.44*	34.90
L.S.D. 5%		0.70	0.67	1.23	1.23	0.48	2.71

*Significant differences compared to the reference control plants.

TABLE 4. Effect of salinization levels and proline or phenylalanine treatments on the content of some nutritive elements in kidney bean roots. Data are expressed as mg/g dry weight.

Amino acid treatments	Salinization level (bars)	Na	K	Ca	Mg	P	N
Reference control	0	1.37	23.18	20.79	12.18	4.99	28.91
	1	6.23	22.37	18.47	11.87	4.10	29.99
	3	17.40	18.14	16.22	12.09	3.50	27.96
	5	27.29	15.62	14.55	11.00	3.41	25.22
Proline	0	1.24	25.49*	21.24	12.43	4.87	30.50
	1	4.75*	22.64	20.49*	11.64	4.40	30.03
	3	13.29*	22.14*	19.18*	12.84	7.08*	31.33*
	5	22.08*	17.60*	18.12*	13.10*	6.95*	28.71*
Phenylalanine	0	1.27	23.95	21.32	12.41	4.84	30.93
	1	4.94*	22.71	19.95*	12.19	4.93*	30.35
	3	13.89*	20.95*	18.85*	12.74	6.13*	31.68*
	5	23.01*	17.02*	18.01*	12.89*	5.82*	29.21*
L.S.D. 5%		1.06	1.40	1.22	0.86	0.39	3.13

*Significant differences compared to the reference control plant

Discussion

In the course of our experiment, we found that salinity induced a significant decrease in the values of the parameters tested (growth, pigment content and mineral composition) of kidney bean.

Reductions in growth parameters (fresh and dry weights) with increasing saliniza-

tion levels were observed by Hutton[2], Shaddad and Heikal[12], and Nerson and Paris[6] working on other glycophytic plants. The inhibitory effect of salinity on growth may be attributed to the effect of NaCl on several facets of plant activities affected by disturbances in osmotic adjustments[18,19], enzyme activities[20], and photosynthesis[21].

The recorded increase in the values of growth parameters of kidney bean induced by the interactive effect between proline or phenylalanine and salinity stress is in accordance with the results obtained by Heikal and Shaddad[11] and Shaddad and Heikal[12] working with other glycophytes. The stimulatory effect of added amino acids may be attributed to the increase in water uptake, thus maintaining hydration of protoplasm[13].

The inhibitory effect of salinity on the biosynthesis of the photosynthetically active pigments is in accordance with the results recorded for other glycophytes[22]. In this connection, Strogonov[23] suggested that the reduction in the pigment content could be probably due to the inhibitory effects of the accumulated ions on the biosynthesis of the different pigment fractions. Moreover, Strogonov *et al.*[24] pointed out that salinity affects the forces binding the complex of pigment-protein-lipid in the chloroplast structure. These structural disorders may account for such differences in pigment biosynthesis. On the other hand, Prisco and O'leary[25] adopted the view that osmotically increased water stress enhances the decay of chlorophyll.

Treatments with either proline or phenylalanine resulted in a considerable enhancement in the biosynthesis of the photosynthetically active pigment. This stimulatory effect is in accordance with the results obtained by Shaddad and Heikal[12], working with maize plants and proline, and Abd El-Samad[26] working on the response of some other glycophytes to the amino acids proline and phenylalanine.

The general increase recorded in sodium content at all salinity levels, is in agreement with the results obtained by Heikal *et al.*[4] working with some oil producing plants. The extent of sodium accumulation with saline solutions varied among shoots and roots of kidney bean plants. In this respect, sodium was more accumulated in roots than in shoots. In accordance with this, Jacoby[27] reported that, in many glycophytes, most of sodium was retained in the roots.

The general reduction in the contents of different minerals (K, Ca, Mg, P and N) in salinized plants is in confirmity with the results obtained by some authors using other plants[4,28].

Spraying with proline or phenylalanine induced a significant reduction in the absorption and accumulation of sodium in shoots and roots of kidney bean at all salinity levels used. This reduction is in accordance with the results obtained by Shaddad and Heikal[12] and Abd El-Samad[26].

On the other side, spraying with any of these two amino acids resulted generally in a significant increase in the contents of K, Ca, Mg, P and N. This promotion in the accumulation of these nutritive elements in the salinized kidney bean plants is in agreement with the results obtained by Shaddad and Heikal[12] and Abd El-Samad[26].

It is worthy to notice that the reduction in the concentration of sodium in kidney bean plants treated with proline or phenylalanine was associated with an increase in phosphorus content. This observation also noticed by Shaddad and Heikal[12] and Abd El-Samad[26]. In this respect, Gates *et al.*[29] stated that the increase in phosphorus contents may be associated with some mechanisms for controlling the salt entering the plant roots. Such regulatory mechanisms would require energy expenditure and phosphorus is usually required for the synthesis of metabolic intermediates necessary for the maintenance of this disequilibrium state.

From our data it can be said that the exogenous application of the amino acids proline and phenylalanine can alleviate the inhibitory effect of salinity on kidney bean plant, within the range studied. However, the role of either proline or phenylalanine in ameliorating the effect of salinity is more complicated and still needs further investigation.

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معادلة التأثير المثبط للملوحة على النمو وبعض الأنشطة الفسيولوجية في نبات الفاصوليا باستخدام كل من البرولين والفنيل ألانين

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المستخلص . استخدمت المزارع الرملية في هذا البحث ، بهدف التعرف على تأثير تركيزات مختلفة من كلوريد الصوديوم (صفر – ٥ بار) على النمو (ممثلا في الوزن الطازج والوزن الجاف) ومحتوى الخضوب النباتية وكذلك تركيز بعض العناصر الغذائية (بو – كا – مغ – فو – ن) في نبات الفاصوليا . بالإضافة إلى ذلك ، تم إجراء تجربة أخرى للتعرف على مايمكن أن يطرأ على هذه التغيرات الحيوية عند رش أوراق نبات الفاصوليا بتركيز معين (١٠٠ جزء في المليون) من الحمضين الأمينيين البرولين أو الفنيل ألانين . وكذلك بهدف التعرف على مايمكن أن تقوم به هذه الأحماض الأمينية لتخفيف أو إزالة الضرر المترتب على نمو النباتات تحت تأثير التركيزات المختلفة من الملوحة .

بينما أحدثت المستويات المختلفة من الملوحة نقصًا جوهريًا في النمو ومحتوى الخضوب النباتية وكذلك تركيز العناصر الغذائية قيد البحث ، أظهر كل من الحمض الأميني البرولين أو الفنيل ألانين تأثيراً فعالاً في خفض التأثير المثبط للملوحة على النمو ومحتوى الخضوب النباتية وتركيز العناصر الغذائية في نبات الفاصوليا ، خصوصًا عند المستويات المتوسطة والمرتفعة من الملوحة (٣، ٥ بار) وذلك بالمقارنة بالنباتات المعاملة فقط بالمستويات المختلفة من كلوريد الصوديوم (نباتات الكنترول) .