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Counteraction of NaCl with NaH_2PO_4 and $NaNO_3$ on pigment, saccharide and protein contents in broad bean

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Abstract

Salinity inhibited growth, and affected the contents of chlorophylls, carotenoids, saccharides, amino acids, proteins, DNA and RNA in broad bean plants. Foliar application of NaH_2PO_4 and $NaNO_3$ greatly ameliorated the adverse effects of NaCl. This counteraction was associated with an increase in contents of saccharides, proteins, DNA and RNA.

Introduction

Plants grown in saline conditions showed marked reduction in their metabolism which is reflected in growth and yield. Salt toxicity may be partly caused by affection of the uptake of essential nutrients (Shaddad 1990). Nucleic acid, protein, amino acids and carbohydrate metabolism rather than pigments and growth are greatly affected in plant grown in saline conditions (Nieman 1965, Malakondaiah and Rajeswararao 1979, Ismail 1991).

Phosphorus and nitrogen are among the key elements of plant metabolism and their uptake by roots is inhibited by salinity (Konda 1947, Bernstein and Ayers 1953, Malakondaiah and Rajeswararao 1970, 1979). Foliar application of nutrients has been found to be more beneficial under saline condition than application of fertilizers to the soil (Malakondaiah and Rajeswararao 1979). Therefore the present work was aimed to study the counteraction of NaCl salinity with the foliar application of phosphorus and nitrogen on broad bean plants.

Material and methods

Broad bean (*Vicia faba* L.) plants were grown in plastic pots in soil without NaCl (control) and under salinization levels corresponding to osmotic potential of NaCl

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solution of -300, -600, -900 and -1200 kPa. Saline solutions were 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 NaH_2PO_4 and $NaNO_3$ (1 g kg^{-1}) solutions were applied three times (5 d intervals) by spraying the shoot system of the growing plants (each pot with 20 cm^3 of NaH_2PO_4 or $NaNO_3$ solutions). The control plants were sprayed with distilled water. A week after the plants were 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). The contents of chlorophylls *a* and *b* and carotenoids were determined spectrophotometrically (Metzner *et al.* 1965), saccharides were determined by the anthrone-sulphuric acid method (Fales 1951). Extraction, separation and estimation of nucleic acids were done according to Schneider (1957) using orcinol reagent. DNA was estimated using diphenylamine reagent (Bartton 1956). Free amino acids were determined according to Moore and Stein (1948) and soluble protein according to Lowry *et al.* (1951).

Results

When broad bean plants were subjected to NaCl, biosynthesis of pigments was markedly activated especially under mild salt stress. Spraying with NaH_2PO_4 or $NaNO_3$ mostly induced no significant changes as compared with the corresponding salinization levels, up to the -900 kPa NaCl, above which, there is a marked increase in these contents, whatever the treatments was applied (Table 1). The fresh and dry masses of bean plants decreased with the rise of salinity in the culture medium. Foliar application of P or N salts resulted, in most cases, in a pronounced increase in the values of fresh and dry masses (Table 1).

Salinity induced a significant decrease in both saccharides and protein contents (Table 2). Treatment with any of the two nutrients resulted in their marked and progressive accumulation. This stimulatory effect was much more obvious in the case of saccharides, whatever the salinity levels used and the plant organ tested.

More amino acids were accumulated with rise of salinity levels in both shoots and roots of broad bean plants. Spraying the shoots with NaH_2PO_4 showed in most cases the same trend as in control plants (Table 1). On the other hand, spraying with $NaNO_3$ mostly resulted in a pronounced reduction in the contents of amino acids whatever the salinity levels used. With the rise of NaCl a depression in contents of DNA at all salinity levels and in RNA at - 1200 kPa was indicated (Table 1). Spraying with $NaNO_3$ mostly resulted in an increase in DNA and especially in RNA as compared with those of the corresponding salinization levels. However, spraying with NaH_2PO_4 did not result in corresponding increase in RNA while in DNA a significant increase was induced (Table 1).

Table 1. Effect of NaCl salinity and foliar spraying with NaH_2PO_4 and $NaNO_3$ on pigment content [g kg^{-1}(d.m)], fresh and dry matter (g $plant^{-1}$), DNA and RNA content [g kg^{-1}(d.m.)] and amino acids [g kg^{-1} (d.m.)] of broad bean plants.

Spraying	NaCl [-kPa]	Chlorophyll *a*	*b*	Carotenoids	Shoot f.m.	d.m.	Root f.m.	d.m.	DNA	RNA	Amino acids Shoot	Root
0	0	6.57	2.56	2.73	25.80	3.13	20.99	1.01	0.410	6.50	25.12	17.18
	300	9.52**	3.48	4.04*	22.28**	2.39**	16.56*	1.06	0.440	7.21	25.98	23.39**
	600	9.22**	3.35	4.14*	20.17**	2.25**	20.65	1.14*	0.402	7.47	27.48**	23.96**
	900	10.15**	3.66*	4.06*	22.06**	2.41**	18.93	1.17**	0.340**	7.43	27.19*	20.51**
	1200	8.44**	3.47	3.75	10.35**	1.13**	10.74**	0.66**	0.322**	4.35	31.56**	20.63**
NaH_2PO_4	0	7.53*	3.19	3.56	24.79	4.90**	21.09	1.37**	0.475**	6.95	25.94	25.21**
	300	7.98*	3.14	3.53	25.03	2.79	23.44	1.22**	0.471**	6.91	28.93**	24.73**
	600	7.61*	3.29	3.28	27.19*	2.93	22.67	1.38**	0.448*	6.36	27.29*	25.33**
	900	7.32	3.44	3.55	16.71**	1.83**	15.59*	1.01	0.401	6.09	27.22*	23.89**
	1200	12.30**	4.64**	5.55**	16.03**	1.84**	11.26**	0.78**	0.347**	6.12	29.20**	25.31**
$NaNO_3$	0	8.44**	2.14	2.59	32.88**	3.68*	24.12	1.52**	0.595**	9.44	10.05**	18.44
	300	8.21*	2.26	2.99	30.80**	3.41	24.67	1.21**	0.607**	10.21	13.80**	19.45*
	600	7.91*	3.92**	3.18	26.84*	3.06	24.82	1.28**	0.503**	8.47	16.72**	18.67
	900	8.97**	3.04	4.01	14.57**	1.33**	25.65*	1.53**	0.462**	8.43	16.66**	20.07*
	1200	13.24**	3.81*	6.42**	14.17**	1.30**	12.41**	0.77**	0.340**	7.35	23.69	19.85*
L.S.D.	5 %	0.87	0.95	1.29	1.04	0.423	4.29	0.109	0.034	3.81	1.73	2.21
L.S.D.	1 %	1.77	1.28	1.74	1.39	0.569	5.78	0.147	0.046	5.13	2.33	2.98

Significance of differences to control: * - $P = 0.01$; ** - $P = 0.05$.

Table 2. Effect of NaCl salinity and treatments with NaH_2PO_4 and $NaNO_3$ (1 g kg^{-1}) on saccharides and protein contents [g kg^{-1}(d.m.)] of broad bean plants.

Spraying	NaCl [-kPa]	Saccharides soluble shoot	root	insoluble shoot	root	Proteins soluble shoot	root	insoluble shoot	root
0	0	14.44	4.65	162.83	45.20	54.5	24.60	68.9	63.1
	300	15.33	6.99*	80.00**	84.82**	40.8**	30.50**	52.1**	60.8**
	600	12.72	6.13	71.83**	57.27**	47.9**	28.50**	66.6**	60.7**
	900	10.11*	7.58**	63.65**	56.46**	58.1**	22.80**	60.1**	37.0**
	1200	6.23**	6.80*	60.11**	45.26	58.2**	21.50**	46.1**	36.9**
NaH_2PO_4	0	37.64**	23.49**	192.90**	132.60**	59.3**	34.70**	125.0**	66.0**
	300	38.74**	38.27**	221.63**	130.30**	58.5**	36.30**	78.6**	53.4**
	600	24.88**	29.56**	221.84**	126.70**	58.1**	36.60**	76.3**	55.4**
	900	25.12**	33.12**	109.84**	98.50**	69.8**	38.20**	86.4**	57.4**
	1200	19.20*	17.59**	86.89**	103.20**	65.6**	31.20**	39.0**	61.2**
$NaNO_3$	0	18.66*	11.77**	245.44**	277.30**	63.4**	39.30**	33.9**	35.8**
	300	21.54**	27.02**	217.80**	202.70**	51.5**	31.90**	42.6**	97.3**
	600	17.96	12.56**	264.82**	152.20**	59.7**	38.50**	41.2**	102.0**
	900	15.55	9.09**	173.19*	152.20**	67.7**	35.20**	37.5**	70.2**
	1200	15.10	9.09**	157.23	79.26**	62.9**	36.60**	30.6**	34.4**
L.S.D	5 %	3.92	2.08	10.34	6.51	1.23	0.401	0.48	0.13
L.S.D.	1 %	5.28	2.80	13.93	8.77	1.66	0.540	0.65	0.18

Significance of differences to control: * - $P = 0.01$; ** - $P = 0.05$.

Discussion

Salinity caused a great reduction in fresh and dry matter production, saccharides, protein, DNA and RNA contents. This was accompanied with a considerable increase in pigment and amino acids contents. The adverse effects of NaCl could be mainly due to its toxicity (Lashaye and Epstein 1971, Munns *et al.* 1979) and/or water deficit (Meiri and Poljakoff-Mayber 1971, Ahmed *et al.* 1980). The observed losses in saccharides as well as in proteins in plants treated with only NaCl were accompanied with a marked and progressive increase in the total free amino acids content, thus salinity may stimulate their conversion into amino acids (Stewart *et al.* 1966) and/or inhibit amino acid incorporation into proteins (Barnett and Naylor 1966, Handa *et al.* 1983, Devitt *et al.* 1987). In addition, the reduction of DNA and RNA levels in the variously salinized broad bean plants might be the primary effect of salinity (Nieman 1965). Thus it can be said that NaCl accelerates the degradation of microsomal and ribosomal RNA and depressed synthesis of proteins (Rauser and Hanson 1966). The recorded promotion in growth after spraying salinized broad bean plants with NaH_2PO_4 or $NaNO_3$ may be due to the marked and progressive increase in soluble saccharides as well as soluble proteins (Eaton 1949, Ergle and Eaton 1957, Ismail 1991) which contribute to osmotic adjustment.

In conclusion, the foliar application of nutrients may be a promising approach for improvement the cultivation of plants under NaCl salinity.

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