

Alleviation of Salinity Stress on Growth and Related Parameters in Wheat Sprayed with Thiamine, Nicotinic Acid or Pyridoxine

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ABSTRACT. The interactive effect of salinity and some vitamins; thiamine, nicotinic acid and pyridoxine on growth and some metabolic changes of wheat (*Triticum aestivum* L.) were studied. Salinity induced a considerable reduction in growth, pigment contents, carbohydrates and proteins in wheat plants. However, proline contents of the main plant organs were increased progressively with the increase of salinity level.

When these salt stressed plants were sprayed with thiamine, nicotinic acid or pyridoxine the growth, pigments and accumulation of carbohydrates and proteins were considerably increased. However, proline contents were decreased as compared with the corresponding treatments with NaCl. These results may lead to the conclusion that, each of the three vitamins used can alleviate the adverse effects of salt stress.

Salinity is a major problem in today's irrigation agriculture, as millions of tons of salts are annually dumped onto the soil from irrigation water. The reduction in growth of many crop plant by salinity may results from salt effects on dry matter allocation, ion relations, water status, physiological processes, biochemical reactions or a combination of such factors (Greenway and Munns 1980, Epstein 1983 and Shah *et al.* 1987). To alleviate the harmful effects of salinity on plant growth and relevant metabolic activities, phytohormones have been used (Singh an Darra 1971, Boucaud and Ungar 1976 and Bejaoui 1985).

Vitamins like thiamine, nicotinic acid and pyridoxine are a special class of plant growth substances superficially similar to phytohormones (Price 1970). Also,

vitamins which can be used in the biosynthesis of coenzymes perform major physiological function in plants (Kefeli 1981). Studies on the direct effect of some vitamins on seed germination appeared quite promising (Polimbetova *et al.* 1964 and Ovcharov and Kulieva 1968). Moreover, presoaking seeds of lupin and broad bean in either ascorbic acid or pyridoxine counteracted the adverse effects of salinity on germination, seedling growth and some metabolic mechanisms (Shaddad *et al.* 1990).

The present work was conducted to study the interactive effect of salinity and the exogenous application of the vitamins; thiamine, nicotinic acid and pyridoxine on growth and some metabolic processes and mineral composition of wheat plants. Wheat was chosen because it is one of the most important grain crops in the world.

Materials and Methods

Seeds of wheat (*Triticum aestivum* L.) were germinated in moist vermiculite until emergence of the primary leaves, at which time equal number of seedlings were transplanted into 30-cm-diameter pots. Containing support media (vermiculite: perlite, 1:1, v/v). Pots were placed in trays containing nutrient, (Hoagland and Arnon, 1950). The plants were grown on this control solution for 10 days, at which time salinization was started. The pots of each of the tested plants were divided into four groups, of four pots and then watered with 1/2 strength Hoagland solution containing various concentrations of NaCl (00, 50, 100 and 150 mM NaCl). Every two days, each group was irrigated with the respective saline solution.

Three other groups of the tested plant were treated with different saline solutions as mentioned above and in addition were sprayed with 100 ppm of each of the three vitamins (thiamine, nicotinic acid and pyridoxine). Two sprays were applied, the first (10 ml. for each pot) after 8 days and the second (15 ml. per pot) after 17 days of salinization. The solution completely covered all leaves, but did not drip. Controlled environment growth chambers were used at $25 \pm 2^\circ\text{C}$ (day/night) on a 14-h day, photosynthetic photon-flux density of $600\text{-}700 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 60-70% relative humidity. All plants used in experiments were maintained at their final salinity for 30 days before use.

Total photosynthetic pigments were determined colorimetrically by the method recommended by Metzner *et al.* (1965). For analysis of proline, carbohydrates and proteins, segments from the three main organs of the plants were immediately frozen in liquid N_2 , lyophilized and weighed. Free proline was determined according to the method described by Bates *et al.* (1973). For

determination of carbohydrate, the anthrone sulphuric acid method (Fales 1951) was used. The microkjeldhal technique was employed total combined nitrogen and soluble protein was determined according to Lowry *et al.* (1951)

Shoots (leaves plus stems) and roots of tested plant were harvested separately and weighed. They were then oven dried at 70°C to constant weight. Portions of dry samples used for determination of Na⁺, K⁺, Ca²⁺ and Mg²⁺ concentrations by atomic absorption spectrophotometry. Phosphorus was determined colorimetrically using the phosphomolybdate method (Jackson 1958).

Three replicates were used and the data were statistically analysed to calculate the least significant difference (L.S.D.).

Results

The data concerning the growth of variously treated wheat plants are given in table 1. Fresh and dry weights of shoots and roots of wheat plants were sharply reduced with the increase of salinity level. Treatment with any of the three vitamins resulted in a significant increase in fresh and dry weights of experimental plant when compared with the corresponding treatments with NaCl.

The contents of the photosynthetically active pigments (chlorophyll a, chlorophyll b and carotenoids) of leaves of test plant harvested 30 days after treatment are given in table 2. It can be seen that generally there were no considerable differences in the pigment fractions and consequently in the total pigment content of the plants subjected to the different levels of salinity. However, the contents of various pigment fraction of plants subjected to 150 mM NaCl were considerably lowered. On the other side, the rate of biosynthesis of each of chlorophyll a and chlorophyll b could be tested using the values of chl.a/chl.b ratios. It can be seen that salinized wheat plants exhibited lower values of a/b ratios than the of control plants.

Spraying wheat plants with any of the three vitamins induced a significant increase in the contents of pigment fractions whatever the level of salinity used. The maximum values of pigment contents were obtained in plants treated with nicotinic acid, while the minimum values were in plants treated with thiamine. Pyridoxine exerted an intermediate effect on the pigment biosynthesis (Table 2). When the values of chl.a/chl.b ratios are taken into consideration. It can be seen that in vitamin treatments these values were generally reduced with rise of salinization level. This mean that the rate of chlorophyll b biosynthesis is not always concomitant with that of chlorophyll a.

The contents of total carbohydrates of variously treated wheat plants are given in table 3. It can be seen that the contents of carbohydrates in the different organs tended to be lowered with the increase of salinity level.

Wheat plants sprayed with any of the three vitamins resulted generally in a significant accumulation of carbohydrates, when compared with the corresponding treatments with NaCl. It can be generally noticed that, when wheat plants were treated with nicotinic acid, the highest amount of carbohydrate were obtained in leaves and stems, but when treated with thiamine, the highest amount of carbohydrates were obtained in roots, under all the level of salinity.

Table 1. Interactive effect of salt stress and vitamine treatments on fresh and dry weights (gm/plant) of wheat plants

NaCl (mM)	Shoots		Roots	
	Fresh weight	Dry weight	Fresh	Dry weight
Control plants				
00	5.40	0.77	1.51	0.55
50	4.51	0.60	1.34	0.48
100	3.95	0.49	1.09	0.38
150	3.82	0.40	0.95	0.32
Nicotinic acid - sprayed plants				
00	7.56*	1.17*	3.58*	1.17*
50	6.39*	0.98*	3.38*	0.86*
100	5.25*	0.74*	3.13*	0.68*
150	4.46	0.55*	1.54*	0.52*
Pyridoxine - sprayed plants				
00	7.97*	1.16*	3.95*	1.31*
50	5.84*	0.90*	3.79*	1.04*
100	4.06	0.69*	2.64*	0.63*
150	3.82	0.56*	1.67*	0.56*
Thiamine - sprayed plants				
00	8.05*	1.04*	3.68*	0.73*
50	6.86*	0.86*	3.06*	0.65*
100	6.21*	0.68*	1.81	0.58
150	3.92	0.47	1.33	0.43
L.S.D. at 1%	0.78	0.13	0.75	0.24

*Significant differences as compared with reference control plants.

The contents of insoluble nitrogen of wheat plant leaves and stems were appreciably decreased by salinity stress, while the soluble nitrogen fraction did not show any significant change, under all salinity levels (Table 4). In the case of roots, the soluble nitrogen was decreased, while insoluble nitrogen was increased with the increase of salinity level.

Spraying wheat plants with any of the three vitamins resulted in a significant increase in the nitrogen fractions contents of different organs when compared with the corresponding treatments with NaCl. It can be noticed that in wheat plant leaves and stems the maximum contents of insoluble nitrogen was recorded to be in plants subjected to 150 mM NaCl after being sprayed with nicotinic acid, while

Table 2. Interactive effect of salt stress and vitamins treatments on photosynthetic pigments (mg. gm dry weight) of wheat plants

NaCl (mM)	Chlorophyll a	Chlorophyll b	Carotenoid	Total pigments	Chl. a/b
Control plants					
00	6.11	3.68	2.01	11.80	1.98
50	5.97	3.70	1.97	11.64	1.61
100	5.91	3.12	1.72	10.75	1.89
150	4.79	2.99	1.69	9.47	1.60
Nicotinic acid - sprayed plants					
00	7.89*	5.47*	2.54*	15.90*	1.44*
50	7.02*	5.29*	2.53*	14.84*	1.32
100	6.61*	4.46*	2.35*	13.43*	1.48*
150	5.44*	3.98*	1.79*	10.15*	1.37*
Pyrodoxine - sprayed plants					
00	7.08*	4.15*	2.56*	13.79*	1.71
50	6.47*	4.15*	2.52*	13.14*	1.56
100	6.33*	3.96*	2.29*	12.58*	1.60
150	5.36*	3.79*	2.30*	11.45*	1.41*
Thiamine - sprayed plants					
00	7.85*	5.55*	2.88*	16.28*	1.41*
50	6.55*	5.32*	2.73*	15.60*	1.23*
100	5.73*	4.38*	2.35*	13.46*	1.31*
150	4.95	4.10	1.75	11.07*	1.21*
L.S.D. at 1%	1.33	1.25	1.01	2.31	0.31

*Significant differences as compared with reference control plants.

in the roots, the highest amounts were recorded in plants at 150 mM NaCl, but after being sprayed with thiamine (Table 4).

Proline which is regarded as an compatible solute and repeatedly recorded to be accumulated in stressed plants was also followed in this investigation (Table 5). It can be seen that there is a general progressive increasing trend in the concentration of proline, in the different organs of test plant, with increasing salinity stress.

Spraying with vitamins resulted in a considerable decrease in the accumulation of proline in the different organs of the test plant, at all salinity levels. The

Table 3. Interactive effect of salt stress and vitamine treatments on soluble carbohydrate contents (mg. gm dry weight) of wheat plants

NaCl (mM)	Leaves	Stems	Roots
		Control plants	
00	102.3	104.0	106.4
50	104.8	109.6	107.2
100	98.4	100.8	100.0
150	97.6	86.2	85.6
		Nicotinic acid - sprayed plants	
00	149.7*	159.8*	117.6*
50	152.8*	168.0*	123.5*
100	123.3*	144.3*	109.3
150	117.6*	120.8*	117.6
		Pyrodoxine - sprayed plants	
00	125.4*	142.2*	115.6*
50	121.7*	128.3*	125.6*
100	116.8*	118.4*	117.6
150	113.9*	115.6*	122.4*
		Thiamine - sprayed plants	
00	128.3*	136.8*	126.4*
50	120.5*	134.4*	139.3*
100	117.8*	124.8*	127.2*
150	112.9*	120.3*	128.8*
L.S.D. at 1%	13.1	9.54	12.10

*Significant differences as compared with reference control plants.

general decrease in proline accumulation after spraying with any of the vitamins means that the injury caused by salinity was somewhat alleviated after vitamin used.

Sodium concentration of shoots (Leaves plus stems) and roots of the test plant was increased significantly and progressively with the rise of salinization level. However, its accumulation was comparatively less in shoots (Table 6) than in roots (Table 7). Contrary to sodium, the concentrations of most of the tested nutritive elements (K^+ , Ca^{2+} , Mg^{2+}) were decreased with the rise in salinization level. On

Table 4. Interactive effect of salt stress and vitamine treatments on nitrogen contents (mg./gm dry weight) of wheat plants

NaCl (mM)	Leaves		Stems		Roots	
	Soluble nitrogen	Insoluble nitrogen	Soluble nitrogen	Insoluble nitrogen	Soluble nitrogen	Insoluble nitrogen
Control plants						
00	5.90	15.78	4.95	17.23	5.35	14.33
50	4.02	16.03	4.22	12.23	3.85	17.39
100	4.72	19.32	4.39	12.32	3.64	16.63
150	5.54	18.03	4.39	13.80	3.22	18.01
Nicotinic acid - sprayed plants						
00	7.48*	22.82*	5.41*	18.43*	6.11*	16.81
50	6.53*	21.65*	5.52*	18.90*	5.09*	19.70
100	6.96*	20.15*	3.95*	21.17*	4.76*	22.12*
150	6.11	22.92*	5.04*	22.79*	3.85*	22.46*
Pyrodoxine - sprayed plants						
00	7.39*	16.82	5.63*	18.59	6.91*	22.55*
50	5.46*	17.82	5.30*	25.77*	4.76*	24.22*
100	6.20*	22.42*	4.93*	22.30*	4.76*	25.39*
150	6.48	20.23*	4.82*	21.34*	5.52*	22.32*
Thiamine - sprayed plants						
00	6.94	16.95	6.00*	19.27*	6.32*	18.48*
50	6.00*	16.88	5.69*	18.82*	5.20*	26.76*
100	6.60*	14.52*	5.20*	20.54*	4.33*	25.75*
150	6.20*	15.74*	6.05*	21.19*	3.98*	28.99*
L.S.D. at 1%	1.2	2.09	0.28	2.27	0.26	3.04

*Significant differences as compared with reference control plants.

the other hand, phosphorus concentration in shoots and roots of tested plant was increased at most of the salinization levels (Tables 6 and 7).

Wheat plants sprayed with either thiamine, nicotinic acid or pyrodoxine accumulated less sodium in their shoots and roots, when compared with the corresponding treatments with NaCl. Moreover, spraying with any of the three vitamins generally induced a significant increase in the concentrations of most of the measured nutrients analysed in the shoots and roots, by comparison with those of control plants. This stimulatory effect was more pronounced in plants sprayed with nicotinic acid than in those sprayed with thiamine and pyrodoxine (Tables 6 and 7).

Table 5. Interactive effect of salt stress and vitamine treatments on proline contents (mg./gm dry weight) of wheat plants

NaCl (mM)	Leaves	Stems	Roots
Control plant			
00	0.50	0.33	0.35
50	0.74	0.37	0.50
100	0.76	0.53	0.49
150	0.93	0.59	0.53
Nicotinic acid - sprayed plants			
00	0.43	0.21	0.30*
50	0.43*	0.24	0.30*
100	0.49*	0.29*	0.44
150	0.50*	0.33*	0.48
Pyrodoxine - sprayed plants			
00	0.32	0.22	0.28
50	0.34*	0.22	0.31*
100	0.40*	0.27*	0.37
150	0.46*	0.30	0.42
Thiamine - sprayed plants			
00	0.25*	0.18	0.28
50	0.29*	0.17	0.40
100	0.30*	0.27*	0.38
150	0.38*	0.34*	0.46
L.S.D. at 1%	0.19	0.22	0.13

* Significant differences as compared with reference control plants.

Discussion

The data obtained here clearly demonstrate that growth was generally lowered by the NaCl concentration. This reduction in growth could be attributed to (a) an osmotic stress due to a lowering of the external water potential, or (b) effects of specific ions on metabolic processes ranging from the absorption of nutrients to enzyme activation or inhibition (Epstein 1983 and Shah *et al.* 1987). Also, Greenway and Munns (1980) emphasized in addition that there is a general retardation of some enzymatic activities due to salt. Thus the observed promotive effect of the vitamins treatments on the growth of salt-stressed wheat plant may result from a reduction, under salt stress of the level of the naturally synthesized vitamins are suppressed such that exogenous application of vitamins remedies this and allows growth promotion.

Table 6. Interactive effect of salt stress and vitamine treatments on mineral composition (mg./gm dry weight) of shoots of wheat plants

NaCl (mM)	Na	K	Ca	Mg	P
Control plants					
00	1.48	32.08	34.38	18.40	4.71
50	6.20	30.46	30.77	17.51	4.92
100	18.88	26.95	26.62	15.60	4.79
150	29.51	23.84	25.26	14.75	3.74
Nicotinic acid - sprayed plants					
00	1.45	34.18*	33.18*	19.01	5.12*
50	5.21*	33.95*	35.18*	19.02*	5.01
100	16.21*	29.51*	32.12*	18.51*	4.90
150	24.32*	29.90*	30.40*	17.40*	4.81*
Pyrodoxine - sprayed plants					
00	1.21	33.88	33.95	18.96	5.03*
50	4.74*	32.24*	31.56	19.14*	5.05*
100	14.48*	29.70*	31.20*	18.23*	4.48*
150	23.06*	27.99*	30.34*	17.39*	4.11*
Thiamine - sprayed plants					
00	1.33	33.07	35.00	19.20	5.31*
50	4.88*	31.37*	31.12	19.16*	4.98*
100	15.41*	29.32*	30.76*	17.69*	5.02*
150	23.89*	27.71*	29.77*	16.68*	4.22*
L.S.D. at 1%	0.84	1.20	1.48	1.00	0.32

* Significant differences as compared with reference control plants.

Spraying salt-stressed wheat plant with the vitamins, thiamine, nicotinic or pyrodoxine resulted in an increase in its pigment contents as compared with the corresponding treatments with NaCl alone. Consequently the transformation of chlorophyll a to chlorophyll b was affected which could be seen from the values of a/b ratio. It is possible that involvement of exogenously applied vitamins in the pigment biosynthesis. Virgin (1965) attributed the reduction in chlorophyll content under salt stress to the inhibition of protochlorophyll synthesis.

The significant reduction induced in the carbohydrate contents of salt stressed wheat plant is in agreement with results obtained by some other authors using other plants (Lee *et al.* 1974 and Handa *et al.* 1983).

Table 7. Interactive effect of salt stress and vitamine treatments on mineral composition (mg./gm dry weight) of roots of wheat plants

NaCl (mM)	Na	K	Ca	Mg	P
Control plants					
00	1.64	27.82	24.95	14.62	4.49
50	7.48	26.84	22.16	14.50	3.69
100	20.88	21.77	19.46	14.24	3.15
150	32.75	18.74	17.46	13.20	3.07
Nicotinic acid - sprayed plants					
00	1.50	29.31*	26.11	15.89*	4.92*
50	6.02*	28.91*	25.31*	15.81*	4.19
100	15.92*	27.31*	24.22*	14.07*	4.02
150	25.52*	22.32*	22.01*	15.90*	3.89*
Pyrodoxine - sprayed plants					
00	1.49	30.59*	25.49	14.92	4.38
50	5.70*	27.17	24.59*	13.97	3.96
100	15.95*	26.54*	23.02*	15.41*	4.52*
150	26.50*	21.12*	21.74*	15.72*	4.12*
Thiamine - sprayed plants					
00	1.52	28.74	25.58	14.89	4.52
50	5.93*	27.25	23.94*	15.29	4.00
100	16.67*	25.14*	22.62*	14.63*	4.18*
150	27.61*	20.42*	21.61*	15.47*	4.92*
L.S.D. at 1%	1.27	1.68	1.46	1.15	0.33

* Significant differences as compared with reference control plants.

Spraying the salt stressed wheat plant with any of the three applied vitamins not only alleviated the inhibitory effect of salinity on the biosynthesis of carbohydrates, but also induced a significant stimulatory effect.

The inhibitory effect of salt stress on nitrogen contents of the shoots and roots of test plant were also recorded by some other authors, for examples (Morilla *et al.* 1973 and Shaner and Boyer 1976). Spraying of stressed plants with any of the three vitamins resulted in an obvious increase in the contents of nitrogen fractions in the shoots and roots of the salt stress wheat plant. This may be due to the activation of specific enzyme system which are responsible for the protein synthesis. Kemble and Macpherson (1954) stated that drought stress could result in proteolysis and interruption of protein synthesis.

The major effects of salt stress on mineral composition of test plant were increases in sodium content, with decrease in potassium, calcium and magnesium contents, however phosphorus content tended to increase with increasing of salt stress (Zidan 1979 and Cramer and Lauchli 1986). Spraying of plants with any of the three vitamins resulted in a pronounced accumulation of K^+ , Ca^{2+} , Mg^{2+} and P. This increase in the contents of ions in the shoots and roots of test plant may also lead to consider that, the exogenous application of vitamins could increase the efficiency of utilization of water upon stress conditions. In this respect, Pitman (1977) recorded a promotion in plant growth, which consequently resulted in higher rates of ions uptake.

The increase in free proline under saline conditions observed in these experiments could be considered as a storage compound since its accumulation could be at the expense of reduced carbohydrates and nitrogen compounds. This conclusion is in accordance with the results obtained by Singh *et al.* (1972) who pointed out that the accumulation of free proline is a metabolic adaptation, which confers survival value, upon stress relief. This conclusion was supported by the case when this salt stressed plant was sprayed with vitamins, the accumulation of proline was considerably decreased, in leaves stems or roots of tested plant analyzed. This reduction in proline accumulation was concomitant with the promotion in growth the test plants. This promotion may lead to conclude that, each of the three vitamins used can alleviate the adverse effects of at least moderate salinity.

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إزالة الضرر المترتب على نمو نبات القمح تحت ظروف ملحية غير ملائمة باستخدام بعض الفيتامينات

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قسم الاحياء - كلية العلوم - جامعة الملك عبدالعزيز
ص.ب: ٩٠٢٨ - جدة ٢١٤١٣ - المملكة العربية السعودية

يهدف هذا البحث إلى التعرف على التغيرات الحياتية لنبات القمح عند معاملته بمستويات مختلفة من الملوحة وما يطرأ على هذه التغيرات الحياتية بعد رشها بتركيز ١٠٠ جزيء في المليون من بعض الفيتامينات (حامض النيكوتين - البيرووكسين - الثيامين) إضافة إلى ما يمكن أن يعكسه البحث من جوانب تطبيقية.

وقد أسفر البحث عن نتائج يمكن إيجازها فيما يلي :-

- ١ - أحدثت الزيادة في الملوحة إنخفاضاً واضحاً في الوزن الطازج والوزن الجاف لكل من المجموع الخضري والجذري للنبات قيد البحث. إلا أنه بعد معاملة هذا النبات بأي من الفيتامينات المستخدمة، ظهرت زيادة واضحة في قيم الوزن الطازج والوزن الجاف لهذا النبات.
- ٢ - أدت زيادة الملوحة إلى إنخفاض في كميات أصباغ البناء الضوئي لأوراق نبات القمح، هذا وقد أدت المعاملة بأي من الفيتامينات إلى زيادة واضحة في كميات الأصباغ.
- ٣ - إنخفضت كميات السكريات والنيتروجين في كل من الاعضاء الرئيسية الثلاثة لنبات القمح مع زيادة الملوحة، كما وقد أدت معاملة النبات بأي من الفيتامينات إلى زيادة واضحة في كميات السكريات والنيتروجين بالمقارنة بالنباتات المعاملة بالملوحة فقط.

٤ - أدت الزيادة في الملوحة إلى زيادة واضحة في كمية البرولين (منظم الاسموزية في الخلية) في كلٍّ من الأوراق والسوق والجذور لنبات القمح إلا أنه بعد معاملته بأيٍّ من الفيتامينات المستخدمة، انخفضت كميات البرولين في كلٍّ من أعضاء النبات الثلاثة.

٥ - أدت الزيادة في الملوحة إلى تغيرات واضحة في التركيب المعدني لكلٍّ من المجموع الخضري والجذري لنبات القمح، حيث زادت بصفة عامة كمية الصوديوم مع زيادة الملوحة، إلا أنه حدث نقص في كلٍّ من البوتاسيوم والكالسيوم والماغنسيوم والفسفور في المجموع الخضري والجذري بالمقارنة بالنباتات المعاملة بالملوحة فقط.