Effect of Sodium Chloride and Sodium Sulfate on Growth, and Ions Content in Faba-Bean (*Vicia Faba*)

تأثير كلوريد الصوديوم وكبريتات الصوديوم في النمو و المحتوى الآيوني لنبات الباقلاء (*Vicia faba* L.)

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Abstract:

The present experiment was conducted to assess the response of faba-bean (*Vicia faba* cv. Local) to sodium chloride and sodium sulfate at two levels of each (3&6dsm^{-1}) and their combinations; 3dsm^{-1} of NaCl +3dsm^{-1} of Na_2SO_4 and 6dsm^{-1} of NaCl +6dsm^{-1} of Na_2SO_4. Five weeks old plants were subjected to salt treatments for periods of 2, 4, and 6 weeks. Plant dry matter of shoot and roots, soluble protein, chlorophyll content, ions content and proline accumulation were determined.

All salt treatments caused significant reduction in all parameters studied. Na_2SO_4 treatments were more detrimental than NaCl treatments. Also, combination of NaCl + Na_2SO_4 at both concentrations were more effective than single salt treatments. Lengthen the duration of exposing plants to salinity resulted in more growth reduction. Total nitrogen, phosphorus, potassium, and calcium content were reduced significantly by all salt treatments. However, sodium content was increased at all salinity levels used. As proline accumulation increased due to different salt treatments, relative water content of plant tissue was decreased in parallel way. From these results, it is suggested that all salinity levels used have adversely effect on faba-bean growth and ions accumulation.

الخلاصة :

اجريت هذه التجربة لغرض معرفة استجابة نبات الباقلاء(الصنف المحلي) للري بماء يحتوي كل من كلوريدالصوديوم وكبريتات الصوديوم بتراكيز3 و6 ديسمنز/ م لكل منهما او التوليفة المكونة من كلوريد الصوديوم زائدا كبريتات الصوديوم بتركيز3 ديسمنز / م او 6 ديسمنز /م لكل منهما.لكل منهما. عرضت النباتات والتي كانت بعمر خمسة اسابيع الى المعاملات الملحية لفترات 2او 4او 6اسابيع. اخذت القياسات المتعلقة بالمادة الجافة لكل من المجموع الخضري والجذور, البروتين الذائب, محتوى الاوراق من الكلوروفيل والمحتوى الايوني للاوراق وكمية البرولين فيها. بينت النتائج ان جميع المعاملات الملحية قد سببت خفضا معنويا في جميع مؤشرات النمو قيد الدراسة. وقد كانت معاملات كبريتات الصوديوم أشد تأثيرا من معاملات كلوريد الصوديوم. كذلك مزج كلا الملحين وبكلا التركيزين كان أكثر ضررا فيما لواستعمل كلا منهما على حدة كما ان اطالة فترة تعرض النباتات للملوحة زاد من الضرر الحاصل للمؤشرات المدروسة. . وكان محتوى الاوراق من النتروجين الكلي والفسفور والكالسيوم والبوتاسيوم قد قل معنويا بتاثير الملوحة . وبالمقابل فان تاثير الصوديوم قد زاد. كما اظهرت معاملات الملوحة زيادة في كمية البرولين متزامنة مع الانخفاض في المحتوى المائي للنسيج النباتي. وعليه يمكن الاستنتاج من هذه الدراسة انه كان للملوحة تاثير عكسي على صفات النمو وتراكم الايونات في الباقلاء .

Introduction

Salinity is one of the most limiting factors on crop productivity and quality in many geographic areas around the world (Greenway and Munns, 1980). Salt stress has multiple negative effects on plant growth and development such as reducing water potential, causes ion imbalance and in sever cases may lead to toxicity. Also, many major other physiological processes such as; photosynthesis, respiration, and ion uptake may be affected to different degrees by salt stress (Meiri et al, 1971; and Ramoliya et al, 2004). Salinity inhibits both nitrogen assimilation and protein synthesis (Cusido et al, 1987) in addition to stunted plant growth.

Studies on chickpeas (Elsheikh and Wood, 1990) and soybean (Singleton and Bohlool, 1984) have been shown that NaCl concentration more than 0.05 mol/L reduced plant growth, depressed N-fixation, and reduced of tissue N content. This depressive effect of NaCl was also observed in peas and faba-bean (Delgado et al, 1994), and in alfalfa (Lakshmi et al, 1974; and Codorilla et al, 1996). Reduced shoot dry mass in chickpeas was obtained at NaCl concentration of equal to or more than 0.05 mol/L (Elsheikh and Wood, 1990) and in peas and faba bean (Delgado et al, 1994). Sodium sulfate has also depressive effect on plant growth and development. Rogers et al, 1998, mentioned that in several regions around the world, sodium sulfate salt is present in concentration higher than that of sodium chloride.

Frequent or permanent plant exposure to salt stress promotes an increase in sodium and chloride ions content and decrease in K^{+1} , Ca^{+2} , P^{-1}, and NO_3^{-1} (Cramer et al, 1991; and Grattan and Grieve, 1992). Rabie, 2005, found that increase levels of NaCl treatment induced significant increase in Na^{+1} and decrease in K^{+1} , Mg^{+2} , and Ca^{+2} levels in shoot system of faba plants. Also, Khan, 2001; Parida et al, 2004; and Ferreira et al, 2001, indicated that high levels of NaCl inhibits the uptake of K^{+1} leading to K^{+1} and other ions deficiency. Inal (2002) found that salt treatment decreased the calcium content of plant, in addition to decrease the uptake of P , N , and Ca. Moreover, Turhan and Atilla (2004), showed that NaCl levels from 500 to 2000 mg/L caused an increase in the content of Na , Cl , Ca , and Mg while decreased K and P in the shoot of strawberry.

Proline plays multiple role in plant stress tolerance. Several plants have been reported to accumulate free proline when they were subjected to environmental stresses (Inal,2002; Karamanos et al, 1983; and Chu et al, 1976). It was reported that proline accumulated in naturally growing stressed plant is in response to water stress or reduced relative water content (Rajagopal et al, 1977; and Singh et at, 1973) as well as under salt and cold stress. Proline accumulation has been shown to be fast, and it is thought to function in salt stress adaptation through protection of plant tissues against osmotic stress and/ or acting as enzyme protector (Berteli et al, 1995). It may also act as macromolecules protector during dehydration (Sanchez et al, 1998). However, a change in relative water content was found as an immediate response to environmental stress (Rajagopal et al, 1977).

The present work was conducted in order to study the effect of NaCl and Na_2SO_4 on growth, proline accumulation and ion accumulation in faba-bean.

Material and Methods

Seeds of *Vicia faba* cv. Local were used , which were obtained from local market. The seeds were immersed in water for 24 hours prior to planting in order to enhance the germination process. Seeds were planted at a rate of five seeds per plastic pot holding three kilograms of soil: peat moss mixture (1:1). The chemical analysis of the soil is shown in table (1). After emergence, seedlings were thinned to two seedlings per pot. Plants were grown under lath house conditions. The temperature was ranged from 32C^o to 15C^{o}during day time and from 18C^o to 11C^{o}during the night for the whole season of growing. Plants were irrigated with tap water for five weeks before applying the treatments. Moisture content was monitored continuously to keep pot soil near the field capacity.

Salt Treatments:

Plants subjected to salt treatments when they were five weeks old. Plants divided into three groups: group one was treated for two weeks with salts, group two was treated for four weeks, and group three was treated for six weeks. Salt treatments included: 3dsm^{-1} of NaCl, 6dsm^{-1} of NaCl, 3dsm^{-1} of Na_2SO_4, 6dsm^{-1} of Na_2SO_4, 3dsm^{-1} NaCl +3dsm^{-1} Na_2SO_4, and 6dsm^{-1} NaCl+ 6dsm^{-1} Na_2SO_4 in addition to control (salt-free). Plants were irrigated again with tap water from the end of each salt treatment until the harvesting time.

Determinations:

Three plants from each treatment were harvested one week after the applying of the six week salt treatments. The above ground parts and roots were taken, cleaned and weighted immediately. Then, they were dried at 60C^o for 24h and weighted again in order to calculate the dry weight.

*Salt Tolerance Index(STI):*STI for each treatment was calculated as fellow (Neera and Ringu, 2004)
STI =(TDW at S_1/ TDW at S_2)X100
Where TDW: Total dry weight (shoot and root)
S_1: for control S_2: for treatment
Soluble Proteins: Soluble proteins were determined according to Lowry et al (1951) method.
Chlorophyll content: After extraction with 80% acetone, the chlorophyll content in leaves was determined according to Arnon (1979).
Relative Water Content (RWC): One inch (2.54 cm) of leaf blade from five leaves was used to determine the RWC using the method described by Weatherley (1950).
Proline Content: Proline content in the leaves were determined using the acid-ninhidrin method (Bates, 1973).
Mineral Content Determination: Proper samples from the dried leaves were prepared for chemical analysis. Total nitrogen was determined using the micro-kjeldahl method (Johnson and Urich, 1975). Potassium and sodium were assayed using flame spectrophotometer (Allen et al, 1984). Phosphorous was extracted and measured spectro-photometrically by using the phosphomolbidate method (Kitson and Mellon, 1944). Calicum was determined according to the method of Johnson and Urich, 1975. All determinations were taken only for the two and four weeks treatments.

Treatments were arranged as a factorial experiment in a completely randomized design with four replications. Treatments means were compared by LSD test ($P<0.05$) (Al-Rawi and Al-Ani, 1990).

Results and Discussion

Dry mass of shoot and roots was decreased significantly ($P <0.05$) by all salt treatments (table 2). As the concentration of sodium chloride or sodium sulfate or their combination increased from 3 to 6dsm^{-1}, dry weight decreased also. The decrease was more pronounced due to Na_2SO_4 treatment. Prolong the exposure time of plants to salts from 2 to 6 weeks accompanied by additional significant decrease in dry weight. Dry weight decreased from 25.26g per plant to18.88g per plant as the time of exposure to salts increased from 2 to 6 weeks respectively.

Interaction between salts and weeks of treatment revealed that the combination of NaCl and Na_2SO_4 at 6dsm^{-1} applied to plants for six weeks gave the least dry weight (14.2 g per plant) while the control treatment gave the highest dry weight (30. 9g per plant). Salt treatment index reflected same results as that for the dry mass (table 3). It decreased significantly by all salt treatments in compare to control. The least index was due to the use of Na_2SO_4 at 6dsm^{-1}.

In accordance with these results it was found that NaCl concentrations from 0.025 mol/L to 0.1 mole/L caused a clear reduction in plant growth of peas and faba-bean *(*Delgado, et al, 1994). Similar results were also found on chickpeas (Elsheikh and Wood, 1990: and Neera and Ranju, 2004). Cheesman (1988) stated that salinity stress imposes additional energy requirements on plant cells to divertes metabolic carbon to storage pools so that less carbon is available for growth.

The data in table. 4 clearly showed that chlorophyll content was significantly reduced in faba plants at all salinity levels used. However, this reduction in chlorophyll pigments might have been responsible for the lower dry matter accumulation in leaves. Combination of NaCl and Na_2SO_4 at 3 dsm^{-1} was the most effective treatment in reducing chlorophyll content. Comparing the treatments with each other, NaCl at 3dsm^{-1} was the least effective treatment in reducing chlorophyll. Also, the results showed further decline in chlorophyll content as the time of exposing plants to salt increases. Interaction of different treatments over weeks of exposure to salts revealed that exposing plants to NaCl plus Na_2SO_4 both at 6dsm^{-1} for six weeks gave the least chlorophyll content. This negative effect of salinity on chlorophyll content has previously been described (Maston et al 1988; and Neera and Ranju 2004). The inhibitory effect of salt on chlorophylls could be due to suppression of specific enzymes responsible for the synthesis of green pigments (Strogonove et al, 1970).

Soluble protein was also decreased significantly by all salt treatments used expect that of NaCl at 3dsm^{-1} (table 5). As the levels of salts or exposing times increased, soluble protein decreased also.

Abd Al-Samad et al (2005) had attributed the less accumulation of protein to the decrease in roots absorbing zones due to the effect of salinity on fresh and dry weight of plant.

Minerals content in plant tissues were significantly influenced by all salt treatments (table 6). The decline in root dry mass, as part of total dry mass, reduce plant's ability to seek nutrients in the soil and transport them to growing shoots. The nitrogen content was significantly reduced by all salinity levels except of NaCl at $3dsm^{-1}$ which did not differ significantly from the control. Meiri et al, 1971, have stated that several physiological processes such as ion uptake and accumulation may be affected to different degrees by chloride and sulfate salts. Nitrogen content was reduced by about 50% when salts introduced to plants as a combination of NaCl + Na_2SO_4 at $6dsm^{-1}$ concentration. However, sodium sulfate at both concentrations was more effective than sodium chloride in reducing N content. This may be explained by the antagonism between Cl and SO_4 (Santamaria et al, 1998). On the other hands, lengthen the exposing time of plants to salinity to four weeks did not effect the nitrogen content of the plant tissues. Phosphours accumulation decreased significantly by all salt treatments. Manchanda et al, 1982, stated that excessive accumulation of chloride in plant tissues inhibit the phosphate uptake. Potassium and calcium accumulation were decreased also by salt treatments with a pronounced effect due to use of Na_2SO_4. Similar results have been shown by Alpasian and Gunes, 2001 and Adams and Ho, 1989. Also, Papadopoulos, 1984, showed that as SO_4 salts present in high concentration in soil or irrigation water, Ca ions precipitate as gypsum and due to low solubility of gypsum this may lead to Ca deficiency. In addition, it should be noted that concentrations of P, K, and Ca in plant tissues were significantly decreased with rising the levels of both salts as well as their combination. Sodium sulfate was more effective in reducing concentrations of these minerals than sodium chloride treatments.

In contrast, sodium content of plant tissues increased significantly by all salt treatments. As the concentration of each salt or their combination increased, sodium content increased also. The highest Na content was reached by the combination treatments. It has been reported by several investigators that salinity caused increased in Na and decreased in Ca and K content (Santamaria et al, 1998; Rulz and Cerda, 1999; and Tavori et al 2004).

Proline has accumulated in plant tissues in response to salinity treatments except for sodium chloride treatment at both concentrations which did not differ significantly from the control (table 7). Treatment with NaCl +Na_2SO_4 at $6dsm^{-1}$ for six weeks gave the highest level of proline in plant tissues. Increase weeks of subjecting plants to salinity caused further accumulation of proline. The increase in proline content and decrease in chlorophyll synthesis results in this study came in accordance with previous results of Soussi et al 1998.

An interesting results regarding relative water content was obtained (table 8). NaCl salt at both concentrations increased RWC in plant tissues. Rulz and Cerda, 1999, indicated that Na, Cl, and NaCl and other macronutrients increased plant tissue water content in two citrus rootstocks. However, plants exposed to Na_2SO_4 salt at both concentrations did not show any changes in RWC , while the combination of the two salts decreased significantly RWC. These results agreed with the results of Inal, 2002, who reported a pronounced decreased in water content of plant tissues due to the treatment with 30mM of NaCl and 15mM of Na_2SO_4. Caldwell, 1976, mentioned that stunned root growth due to salinity could lead to decrease tissue water content. Also, it was suggested that K^+ and Cl elements to be the potential target on osmosensing in the stomatal guard cells (Lin and Luan, 1998) and because K^+ cation transport to plant tissues causing shoot osmoregulation, this may in turn decrease water flow and then growth improvement (Marschner, 1995). It is worthy to notice that as the proline accumulated in plant tissues , RWC decreased by the same manner.

Overall, it may be concluded that both NaCl and Na_2SO_4 inhibit growth, metabolic activity, and ion uptake. However, Na_2SO_4 affected plant growth more adversely than NaCl. Combination of the two salts were more effective than single salt treatment. In addition, prolong the duration of time to which plant exposed resulted in more negative effect. Ion accumulation decreased by all salt treatments except Na content which increased dramatically with the increasing of salt concentrations.

Table(1) Physical-chemical properties of the soil of the experiment.

Property	Value
Texture	Silt 43% Clay 33% Sand 24%
EC	1.2dsm^{-1}
PH	7.6
Total organic carbon (%)	1.1
Total nitrogen(%)	0.09
Soluble ions (mg/L)	SO_4^{-1} 25.2, Cl^{-1} 6.0, Na^{+1} 0.40, K^{+1} 16.5, Ca^{+2} 11.8, P^{-1} 6.1

Table (2).Effect of sodium chloride and sodium sulfate salinity on total dry weight (g/ plant) of faba bean plant.

Salt Treatment (dsm^{-1})	Weeks of Treatment			Treatment Mean
	2	4	6	
Control				30.90
NaCl (3)	29.7	25.0	22.4	25. 70
NaCl (6)	29.0	23.0	21.7	24.50
Na_2SO_4(3)	26.2	25.0	21.1	24.10
Na_2SO_4(6)	23.6	20.3	15.6	19.83
NaCl (3) + Na_2SO_4(3)	23.0	21.5	18.3	20.93
NaCl (6) + Na_2SO_4(6)	20.1	16.5	14.2	16.93
Mean	25.26	21.88	18.88	

LSD 5% for: Salts 4.4 Weeks of treatments 4.1 Interaction 6.4

Table (3) Effect of sodium chloride and sodium sulfate salinity on salt tolerance index (STI) of faba bean plant.

Salt Treatment (dsm^{-1})	Weeks of Treatment			Treatment Mean
	2	4	6	
Control				100.00
NaCl (3)	96.11	80.90	72.49	83.16
NaCl (6)	93.85	74.43	70.22	79.50
Na_2SO_4(3)	84.78	80.90	68.28	77.98
Na_2SO_4(6)	76.37	65.69	50.48	64.18
NaCl (3) + Na_2SO_4 (3)	74.43	69.57	59.22	67.74
NaCl (6) + Na_2SO_4(6)	65.04	53.39	45.95	54.78
Mean	81.76	70.81	61.10	

LSD 5% for: Salts 10.4 Weeks of treatments 10.2 Interaction 14.1

Table (4).Effect of sodium chloride and sodium sulfate salinity on chlorophyll content ($mg.g^{-1}.dw$) in leaves of faba bean.

Salt Treatment (dsm^{-1})	Weeks of Treatment			Treatment Mean
	2	4	6	
Control				740.00
NaCl (3)	650	610	610	623.33
NaCl (6)	690	550	540	593.33
Na_2SO_4(3)	580	500	480	520.00
Na_2SO_4(6)	510	480	380	456.66
NaCl (3) +Na_2SO_4(3)	500	505	340	426.66
NaCl (6) +Na_2SO_4(6)	480	490	320	511.38
Mean	568.33	520.83	445	

LSD 5% for: Salts 45.5 Weeks of treatments 31.8 Interaction 63.3

Table (5).Effect of sodium chloride and sodium sulfate salinity on soluble protein ($mg.g^{-1}.dw$) of faba bean plant.

Salt Treatments (dsm^{-1})	Weeks of Treatments			Treatment Mean
	2	4	6	
Control				41.5
NaCl (3)	42.2	40.1	41.4	41.23
NaCl (6)	38.3	36.5	32.2	35.70
Na_2SO_4(3)	36.2	33.3	30.2	33.23
Na_2SO_4(6)	31.8	30.2	27.5	29.83
NaCl (3) + Na_2SO_4(3)	28.2	26.5	25.0	26.56
NaCl (6) + Na_2SO_4(6)	26.2	22.8	23.3	24.1
Mean	33.81	31.5	29.93	

LSD 5% for:	Salts	Weeks of treatments	Interaction
	5.5	4.2	6.6

Table(6) Effect of sodium chloride and sodium sulfate on ion content in plant tissues of faba-bean treated for 2 and 4 weeks..

Salt treatment (dsm^{-1})	Nitrogen ($mg.g^{-1}$ dw)			Phosphorus (mg.g-1 dw)		
	2weeks	4 weeks	Mean	2 weeks	4 weeks	Mean
Control			14.8			2.33
NaCl (3)	14.4	14.4	14.4	1.76	1.45	1.6
NaCl (6)	12.2	11.8	12.0	1.42	1.43	1.42
Na_2SO_4 (3)	10.4	11.8	11.1	1.21	0.99	1.09
Na_2SO_4 (6)	9.8	10.1	9.95	1.09	0.68	0.97
NaCl (3) +Na_2SO_4 (3)	8.5	7.5	8.0	0.96	0.98	0.97
NaCl (6)+ Na_2SO_4 (6)	7.5	7.4	7.45	0.70	0.64	0.66
Mean	10.46	10.5		1.19	1.05	

Potassium ($mg.g^{-1}$ dw)			Sodium ($mg.g^{-1}$ dw)			Calicum ($mg.g^{-1}$ dw)		
2weeks	4 weeks	Mean	2weeks	4weeks	Mean	2weeks	4 weeks	Mean
		13.3			1.2			5.5
12.1	10.1	11.1	8.4	11.12	9.76	4.2	4.4	4.3
10.4	9.9	10.15	10.2	13.4	11.8	3.6	3.0	3.3
9.8	8.0	8.9	9.3	10.5	9.9	2.9	2.4	2.65
8.6	8.1	8.35	9.8	11.2	10.5	2.3	2.0	2.15
6.4	6.0	6.20	11.4	13.2	12.3	2.3	1.7	2.0
5.7	4.5	5.10	12.15	13.6	12.87	2.1	2.1	2.1
8.83	7.76		10.2	12.17		2.9	2.6	

LSD 5% for:	Salts	Weeks of treatment	Interaction
Nitrogen	2.7	N.S	3.6
Phosphours	0.27	N.S	0.45
Potassium	4.1	0.53	5.2
Sodium	3.3	1.1	2.1
Calicum	1.5	N.S	1.9

Table (7).Effect of sodium chloride and sodium sulfate salinity on proline content ($mg.g^{-1}$. dw) of faba bean plant.

Salt Treatment (dsm^{-1})	Weeks of Treatment			Treatment Mean
	2	4	6	
Control				1.18
NaCl (3)	1.09	1.19	1.20	1.16
NaCl (6)	1.10	1.32	1.33	1.25
Na_2SO_4(3)	1.23	1.48	1.60	1.43
Na_2SO_4(6)	1.33	1.55	1.82	1.56
NaCl (3) + Na_2SO_4(3)	1.30	1.50	1.56	1.45
NaCl (6) + Na_2SO_4(6)	1.30	1.62	1.89	1.60
Mean	1.22	1.44	1.58	

LSD 5% for: Salts Weeks of treatments Interaction
0.23 0.20 0.36

Table (8).Effect of sodium chloride and sodium sulfate salinity on relative water content(%) of faba bean plant.

Salt Treatment (dsm^{-1})	Weeks of Treatment			Treatment Mean
	2	4	6	
Control	70.5			
NaCl (3)	75.2	75.4	78.8	76.46
Nacl (6)	77.6	75.7	80.25	77.85
Na_2SO_4(3)	70.7	69.9	65.20	68.6
Na_2SO_4(6)	72.4	72.7	70.4	71.83
NaCl (3) + Na_2SO_4(3)	65.4	61.4	64.7	63.83
NaCl (6) + Na_2SO_4(6)	65.2	60.9	60.3	62.13
Mean	71.08	69.33	69.93	

LSD 5% for: Salts Weeks of treatments Interaction
4.5 N.S 6.1

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