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Effects of Exogenous Application of Diamine (Putrescine) on Growth and Mineral Elements Distribution in Faba Bean Plants Under Saline Conditions

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□ ABSTRACT □

The interactive effect of NaCl salinity and putrescine on faba bean growth and ion (Na^+, K^+, and Cl^-) distribution in roots and shoots was studied. Salinity decreased transpiration, shoot and root dry matter of faba bean plants. Putrescine application on the leaves or to the root medium, did not affect neither transpiration rate nor dry matter in the control or saline conditions.

Ion distribution in roots and shoots was changed with putrescine treatment: Na^+ and Cl^- ion contents decreased in the shoots of salt stressed plants. K^+ content increased in the roots and shoots of unstressed treated plants, but was not affected under saline conditions. The decrease of Na^+ and Cl^- contents in putrescine treatments under saline conditions and the increase of K^+ content under control conditions did not improve plant growth, this suggests that Na^+ and Cl^- content in the salt stressed plants was not the limiting factor of growth and the same is true for K^+ contents in control plants.

Key words: Salinity, Polyamines, Putrescine**.**

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تأثير المعالجة بثنائي الأمين (Putrescine) في نمو نباتات الفول، وتوزع محتواها من العناصر المعدنية في ظروف الملوحة

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□ الملخّص □

تمت دراسة التأثير المتبادل بين الملوحة (NaCl : 100 mM) والـ Putrescine في نمو نباتات الفول، وتوزع محتواها من(Na^+, K^+ والـ Cl^-) في كل من الجذور والفروع.

خفضت الملوحة النتح والمادة الجافة في جذور نباتات الفول وفروعها، ولم يؤثر تطبيق الـ Putrescine، رشاً على الأوراق، أو مضافاً إلى وسط الجذور، في معدل النتح، أو في المادة الجافة عند النباتات المعالجة، أو غير المعالجة بالملوحة.

أدت المعاملة بالـ Putrescine إلى تغيير توزع الأيونات في الجذور والفروع، فقد انخفض محتوى الفروع من الـ Na^+ والـ Cl^- في النباتات المجهدة ملحياً، وأدت هذه المعاملة إلى ازدياد محتوى الـ K^+ في جذور النباتات غير المعاملة بالملوحة وفروعها، لكنه لم يتأثر في ظروف الملوحة.

إن انخفاض محتوى الـ Na^+ والـ Cl^- ، نتيجة المعالجة بالـPutrescine ، في ظروف الملوحة، وارتفاع محتوى الـ K^+ في ظروف الشاهد، لم يحسن نمو النبات، وهذا يفترض أن محتوى الـ Na^+ والـ Cl^- في النباتات المجهدة ملحياً ليس هو العامل المحدد للنمو، وكذلك الأمر بالنسبة لمحتوى بالبوتاسيوم.

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I. Introduction:

Polyamines are considered as type of plant growth regulators or hormonal second messengers (Galston, 1983). They are ubiquitous in higher plant cells and involved in many plant growth and development processes (Slocum et al., 1984; Smith, 1990). These polycations have been shown to influence membrane permeability (Naik and Srivastava, 1978), cell division, morphogenesis, senescence and response to stress (Slocum et al., 1984; Smith, 1990). This is probably due to their ability to associate non- specifically with cellular macromolecules negatively charged such as phospholipides (Cohen, 1971). Their attachment with phospholipides results in stabilized cell membrane and in altered pattern of solute permeation through the membrane, like the decrease of betacyanine efflux from the discs of beet root storage tissues (Naik and Srivastava, 1978; Srivastava and Smith, 1982a).

Several studies have demonstrated that polyamines retard chlorophyll loss and prevent the rise of RNase and protease activity (Altman,1982; Cohen et al., 1979; Kaur-Sawhney and Galston, 1979).

Contradictory results have been reported on polyamines accumulation under salt stress conditions. They demonstrated that no accumulation of spermine, un-increase of spermidine and a decrease of putrescine contents in rice plants under salt stress (Chaun Chi Lin and Ching Huei Kao, 1995), high accumulation of putrescine under non-ionic osmotic and salt stress in salt and drought tolerant wheat varieties (Erdei et al,1990). The variation of polyamines contents under different stress conditions has suggested un adaptive role for these compounds (Smith, 1985).

There are few reports on exogenous application of polyamines, especially putrescine, indicating their beneficial role in salt tolerance in rice (Parakash and Prathapasenan, 1989; Krishamurthy, 1991; Chattopaddhayay et al., 2002), and mustard (Mishra and sharma, 1994). Foliar application of putrescine (10^{-5} M) significantly increased growth of salt-stressed rice and decreased the influx of Na^+ and Cl^- ions, and increased K^+ content in all tissues of the salinized plant examined (Parakash and Prathapasenan, 1988; Krishamurthy, 1991). Similar results have indicated that polyamines prevented the uptake of Na^+ and loss of K^+ and leakage of amino acids and electrolytes from rice salt-stressed tissues (Chattopaddhayay, 2002). Contradictory reports demonstrated that pretreatment of rice seeds with putrescine caused unincrease in putrescine concentration in shoots, but could not alleviate the inhibition of NaCl on seedling growth (Chaun Chi Lin and Ching Huei Kao, 1995). Cohen et al., (1979) reported that polyamines treatment of barley leaf discs declined the activity of photosystem I and II in the chloroplast, and resulted in a destruction of their envelope.

Chatterjee et al., 1988 have shown an inhibitory effect of polyamine on photosynthesis of source and sink organs in rice.

In the view of the contradictory reported results, they present a point of investigation for the effect of putrescine on growth and mineral elements uptake and distribution in faba bean plants under NaCl stress conditions.

Aim of Study:

This work aimed to test the possibility of alleviating the deleterious effects of NaCl stress on growth of faba bean plants by using some growth substances like diamine (Putrescine) and the reflection on ion distribution in plant parts and plant growth under non-saline and saline conditions.

II. Materials and methods:

Faba bean (Vicia faba L.minor, v. Troy) seeds were germinated in sand imbibed with 1 mM $CaSO_4$, in the Institute of Plant Nutrition, Jutus Liebig university, Giessen, Germany. After 8 days, faba bean seedling were transferred to a hydroponics system (4 plants per pots filled with 5 litres of one-forth strength nutrient solutin). The strength of the nutrient solution was daily increased by one-forth untill the full concentration was achieved. The full-strength nutrient solution contained in mM:

K_2SO_4 ,1; K_2HPO_4 , 0,1; KH_2PO_4 , 0,1 ; KCl , 0,2; $MgSO_4,7H_2O$, 0,5; $Ca(NO_3)_2,4H_2O$,2; in µM: FeEDTA,100 $MnSO_4,H_2O$, 0,5; $CuSO_4,5H_2O$, 0,2; $ZnSO_4,7H_2O$, 0,5; H_3BO_3 ,10; $(NH_4)_6Mo_7O_{24},4H_2O$, 0,05; $CoCl_2,6H_2O$, 0,5; $NiSO_4,6H_2O$, 0,01.The pH was kept at 6.0 (± 0.1).

The experiment was carried out in a growth chamber on the 20th of september 2002. The average temperature during growing time was 24 ºC at day and 16 ºC at night.

After full nutrient solution was achieved, salinization was imposed daily by an increment of 25 mM sodium chloride solution untill a final concentration of 100 mM / l. per pot was achieved. This nutrient solution was changed twice a week.

putrescine (0,1mM) was added in the same time of NaCl application either by addition to

nutrient solution adding to the roots medium (R); or by Spraying on the leaves (L) + 50µl of Teen 20. Therefore, there were 6 treatments, 3 plants by treatment and 4 replicates for each treatment.

Plants were harvested 10 days after the salt treatment were imposed. Roots were washed for 5 min. in bidestilled water followerd by washing in 1 mM $CaSO_4$, and shoots were washed with bidestilled water. Thereafter, shoots and roots system, were dried at 80 ºC for 48 h and dry weights were recorded.

Sub samples of dried and ground shoots and roots plant materials were ashed at (500 ºC) and the ash was digested in 2 ml HCl 6 N …etc. (ADAS, 1986). Then, Na^+ and K^+ concentrations were determined in the digested solution and measured by atomic absorption spectrophotometer.

Chloride concentrations were determined by chloride titration in hot water extracts (Eppenddorf 6610).

Data were statistically analyzed using general analysis of variance (ANOVA procedure) for the effect of salinity (NaCl) , putrescine application (put) and their combination (NaCl × put). Values for standard error were also calculated (SAS Institute 1999)

III – Results and discussion:

1- Transpiration:

Transpiration of faba bean plants grown in nutrient solution with 100 mM NaCl was decreased significantly as compared to the control (fig 1). This is in agreement with data obtained by Delfine et al. (1998) in spinach. The reduction in stomatal conductance was not related to the turgor in spinach (Robenson et al. 1983), or wheat (Kingsbury et al. 1984).

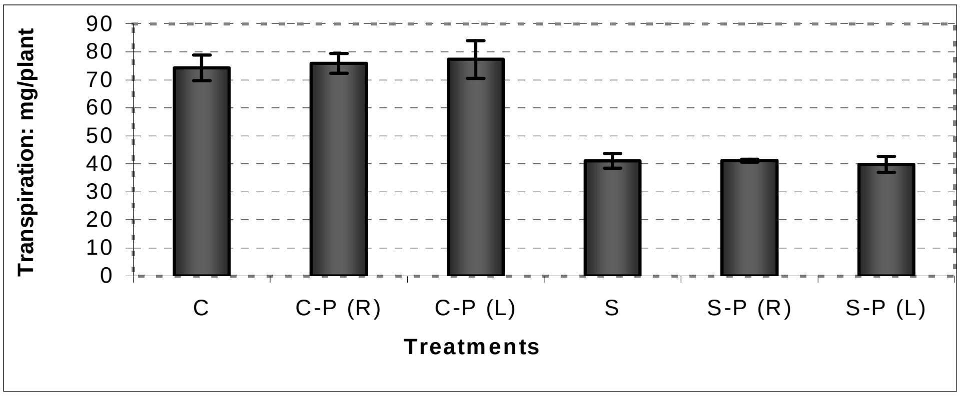

Fig (1): Effect of putrescine application way on transpiration (R: added to the root medium), (L: sprayed on shoots), under saline conditions ± SE.
(NaCl: P< 0.0001, Put: P< 0.97, Salt×Put:P< 0.87 at 5%)

The observed increase in water potential and turgor in wheat and barley leaves did not increase transpiration rate, Teramat et al. (1985). This suggest that a signal from root with low water potential is probably regulating stomatal conductance.

Elevated level of abscisic acid (ABA) are typical responses to water deficit induced by salinity (Marschner, 1986), that cause stomatal closure. However, treatment with putrescine (put) did not change transpiration rate irrespect of NaCl, which indicates that putrescine had no effect on leaf water potential or abscisic acid content in plants.

2- Shoots and Roots dry matter:

Shoots and roots dry matter decreased significantly after 10 days of exposure to 100mM NaCl. The reduction in shoots dry matter was higher than roots dry matter regardless of putrescine treatment (Fig 2).

Application of putrescine (put) to leaves or roots did not improve shoots or roots dry matter under control or saline conditions. The decrease in dry matter of salt treated plants is probably a consequence of growth rate decrease (Amzallag, 1997; Grattan and Greive, 1994; Munns and Termaat, 1986; Ho and Adams, 1994; Prakash and Prathpasenan, 1988-1989).

Some authors reported that the effect of salinity on growth could be due to the inhibition of photosynthesis rate (John et al. 1985), or to the damage of membrane integrity by NaCl (Greenway and Munns, 1980). Other authors contributed the decrease in growth rate to a hormonal signal sent by roots to shoots under salt stress such as abscissic acid (ABA) which according to Wu et al.(1993) modulate the expression of an enzyme affecting plasticity of cell wall, and as a consequence of cell wall expansion.

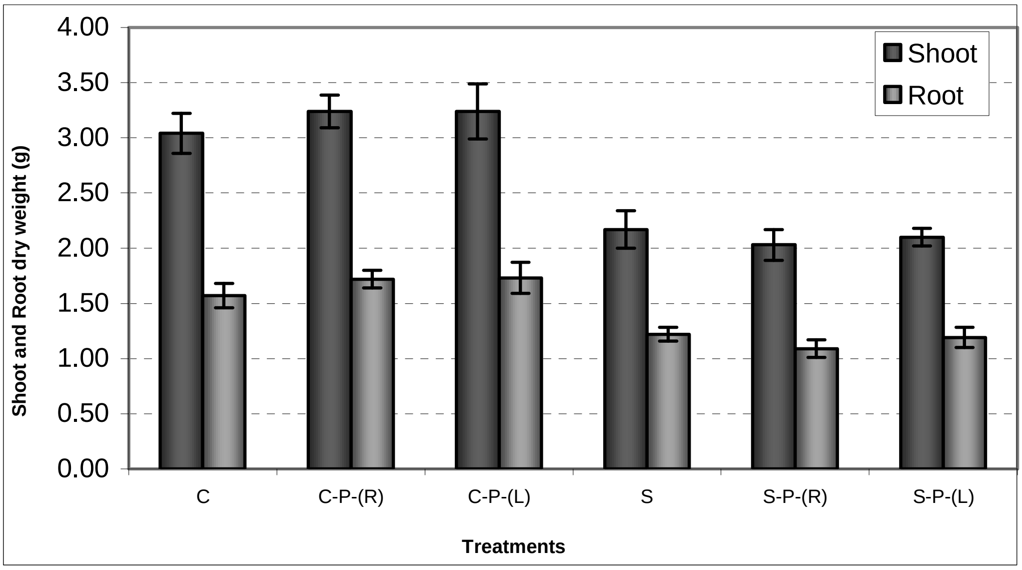

Fig (2) : Effect of putrescine on shoot and root dry weight under salt conditions ± SE. NaCl: P< 0.0001, (Shoots: Put: P< 0.92, Salt×Put:P< 0.57; Roots: Put: P< 0.80, Salt×Put:P< 0.36 at 5%)

Contradictory finding were however reported for rice and mustard plants in which putrescine was reported to alleviate the inhibitory effect of NaCl (Prakash and prathpasenan, 1989; Krishnamurthy, 1991; Mishra and Sharma, 1994). The absence of significant effect of putrescine on faba bean dry matter under non-saline and saline conditions could be due to its ineffectiveness on ABA production or hormonal balance in these plants.

3- Ions content in roots and shoots:

The content of Na^+ in the roots and shoots of stressed faba bean plants increased significantly with NaCl treatment (fig 3). The increase in Na^+ content of shoots was higher than in roots in NaCl stressed plants compared with control plants (fig 4).

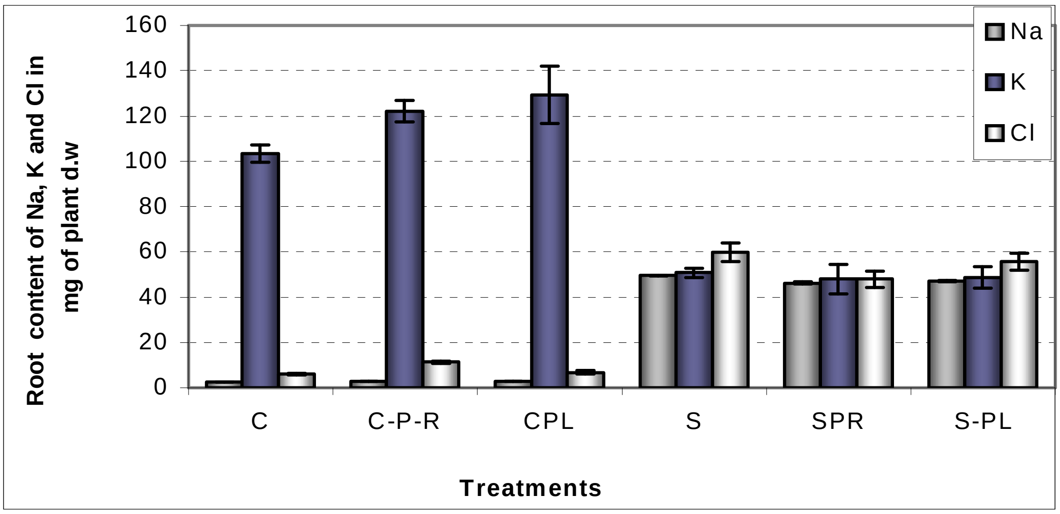

Fig (3): Effect of putrescine on ion content in roots under salt conditions ± SE. NaCl: P< 0.0001, (Na: Put: P< 0.95 , Salt×Put P< 0.43 ; K: Put: P< 0.22 , Salt×Put P< 0.12; Cl: Put: P< 0.53 , Salt×Put P< 0.003 at 5%)

In non-saline treatment, roots accumulated more Na^+ than shoots, whereas, under saline conditions, shoots accumulated more Na^+ than roots (Fig 3 - 4). These results are in agreement with the finding of Assha Yaha (1998) for sesame plants.

Treatment with putrescine did not affect Na^+ content neither in roots nor in shoots under control conditions. Under saline conditions, putrescine did not alter Na^+ content in roots but was decreased significantly in shoots, which suggests that putrescine may inhibit Na^+ transport to the shoots. Other polyamines (spermedine, spermine) were reported to have similar effects (Chattopadhayay et al., 1991; Prakash and prathapasenan, 1988a).

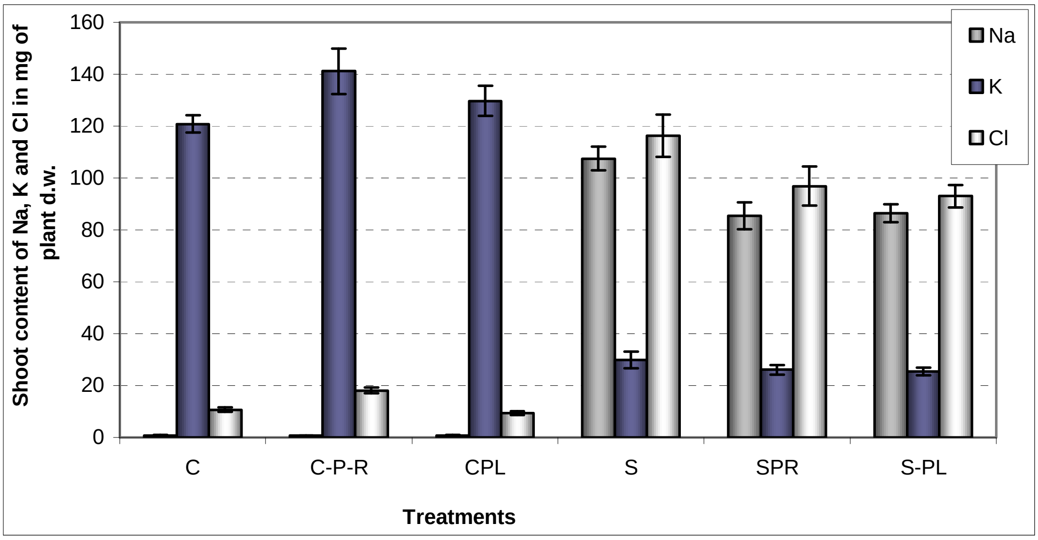

Fig (4): Effect of putrescine on ion content in shoots under salt conditions ± SE.
NaCl: P< 0.0001, (Na: Put, P< 0.007, NaCl ×Put: P< 0.007; K: Put P< 0.19, NaCl ×Put: P< 0.05 ; Cl: Put, P< 0.07, , NaCl ×Put: P< 0.033 at 5%)

K^+ content decreased significantly with salt application both in roots and shoots, which was more pronounced in shoots compared to roots. These results are in agreement with findings for rice (Prakash and Prathapasenan,1988a); and for spinach (Suleiman et al., 2002) in which treatment with NaCl decreased K^+ content in plant tissues. On the other hand, Krishnamurthy (1991) reported an increased K content in NaCl- stressed rice plants.

Ptrescine application to leaves significantly increased K^+ content in roots and when added to the root medium significantly increased K^+ content in shoots in unstressed conditions. Whereas, under saline conditions, putrescine had no effect on K^+ content neither in roots nor in shoots.

The increase of K^+ content in roots and shoots with putrescine treatment under control condition did not improve plant growth, and the absence of putrescine effect under saline conditions suggests that putrescine may enhanced some mechanisms of K^+ absorption which could be inhibited by NaCl.

Cl^- content, was also increased in roots and shoots of salt stressed plants. In contrast of the 2 precedent elements (Na^+ and K^+), Cl^- content was not affected by putrescine treatment under control conditions neither in roots nor in shoots. Under saline conditions, putrescine decreased significantly Cl^- content in roots when it was added to the root, and in shoots when was added in either way. Similar results were reported for rice(Prakash and Prathapasenan 1988a); Krishnamurthy (1991), but in contrast of Suleiman et al. (2002) results in spinach. It could be concluded that the inhibition of Na^+ and Cl^- content in the shoots due to the application of putrescine did not improve plant growth in saline conditions , this would suggests that the decrease in growth rate is rather due to a change in hormonal balance of stressed plants. It seems that putrescine could not alter this process even if Na^+ and Cl^- content in plant tissues were decreased.

Conclusion:

Plant growth decreased by NaCl stress. Putrescine application changed ion distribution in root and shoot of faba bean plants. The reduction of Na and Cl content in faba bean stressed plants and the increase of K content in control plants did not improve plant growth ; This suggest that ion content is not a limiting factor for plant growth , and probably due to a hormonal factor.

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