EFFECTS OF EXOGENOUS ARGININE AND URIC ACID ON *ERUCA SATIVA* MILL GROWN UNDER SALINE CONDITIONS

By:

M. I. Yagi* and S. S. Al-Abdulkareem

Botany Department, Science College, Dammam Girls Colleges, PO Box 838, Dammam, 31113, Saudi Arabia. * P.O. Box 6789, P.C. 11113 Khartoum, Sudan.

Keywords: Erua sativa, salinity alginine, uric acid, ascorbic acid and stress tolerates.

ABSTRACT

Seeds of *Eruca sativa* Mill were soaked in distilled water, NaCl or NaCl with uric acid or arginine or in one of the amino acids. Treatment of seeds of *Eruca sativa* with arginine and uric acid under saline conditions increased the percent of seed germination from 60% to 80%. Incorporation of these amino acids in the nutrient medium also significantly enhanced the dry weights as well as the contents of chlorophyll and ascorbic acid in the seedlings. Levels of both total amino acids and proline were higher in salinized than in non-salinized plants. The results strongly suggest that incorporation of exogenous amino acids in seeds and seedlings of *E.sativa* helps to alleviate salt stress in the species.

الملخص:

تم نقع بذور الجيرجير في الماء المقطر أو كلوريد الصوديوم أو محلول كلوريد الصوديوم مع حمض يوريك أو حمض أرجنين أو في أحد الحمضين الأمنيين. إن معاملة بذور الجيرجير تحت الظروف الملحية أدت إلى ارتفاع في النسبة المئوية للإنبات من 60% إلى 80%. كما أن استخدام هذه الأحماض في الوسط أدى إلى زيادة واضحة في كل من الأوزان الجافة ومحتويات كل من الكلورفيل وحمض اسكوربيك في البادرات. أن مستويات الأحماض الأمينية الكلية وحمض برولين كانت مرتفعة في النباتات المعاملة بالملح عن غير المعاملة. توضح النتائج أن معاملة بذور الجيرجير بحمض ارجنين أو حمض يوريك تقلل من أثر الإجهاد الملحي.

INTRODUCTION

Salinity stress in plants retards all major growth processes that have been examined. Such processes as cell division and enlargement, production of proteins and nucleic acids, energy metabolism and increase in plant mass are adversely affected (Rain 1972; Mass and Neiman 1978; Greenway and Munns 1983; Aspinall 1986; McCree 1986; Basalah 1991). Since non - halophytes have no means of salt tolerance regulation**,** ion build up takes place in their leaves if salinized. Consequently, growth of such plants is curtailed and death ensues (Munns and Termaat 1986; Flowers and Yeo 1986). (Greenway and Munns 1980) pointed out that more tolerant non **–** halophytes avoid ion excess, but may consequently be deficient in solute for osmotic regulation. Water deficits may exert their effects directly on cell extension and division (Greenway *et al.* 1983) .However, different plants respond differently; for instance exposure of cotton (*Gossypium hirsutum*) and bean (*Phaseolus vulgaris*) plants to various levels of salinity stress reduced leaf area and caused decrease in total respiration (Hoffman and Phene 1971). Likewise, McCree *et al.* (1984) observed inhibition of sorghum (*Sorghum bicolor*) leaf expansion under salt stress. Irrigation of faba beans (*Vicia faba*) with saline water reduced plant heights, green matter, and dry weights (Fallatah and Hussain (1988). Prakash *et al.* (1988) found out that decrease in growth of rice (*Oryza sativa*) caused by NaCl was concomitant with decreases in the contents of nucleic acids and protein. Chlorophyll contents were also reduced under salinity conditions in rice (Yeo and Flowers 1986) and wheat (Zidan 1991) plants. Hale and Orcutt (1987) concluded that the majority of crop plants, including leafy vegetables, are susceptible to salinity. They apparently either do not survive high salinity conditions or, at best, do so with decreased yield.

Attempts to alleviate the adverse effects of salt stress in non-halophytes are meager. Presoaking of wheat (*Triticum aestivum*) seeds in plant hormones before subjection to salinity increased nutrient uptake and yield (Balki and Padole 1982). Zidan (1991) has also found out that certain vitamins reduced the deleterious effects of sodium chloride on wheat growth. The present work is an attempt to seeking means of improving the survival of *Eruca sativa* Mill under experimental saline conditions.

MATERIALS AND METHODS

Seeds of garden rocket (*Eruca sativa* Mill) were surface sterilized with 2% sodium hypochlorite for 3minutes. They were then thoroughly washed with distilled water and left to dry overnight. Subsequently, seeds were soaked for 6hrs in one of the following: Distilled water, 100 mM NaCl, 100 mM NaCl + 10 mM uric acid, 100 mM NaCl + 10 mM arginine, 10 mM uric acid and 10 mM arginine.

After that, they were sun dried for 8hrs. Soaking and sun drying was repeated twice. Batches of seeds were germinated in Petri dishes containing ash less filter paper moistened with a solution identical to that they had originally been soaked in. The percent of germination was recorded after 5days. Seedlings were selected on homogeneity of height and developmental stage, and transferred to plastic pots (5 seedlings/pot) containing vermiculite moistened with the same soaking solution but containing quarter strength of Hoagland's solution. Seedlings were then raised in a greenhouse under natural light. Temperatures were 25-28˚C day, 19-23˚C nights with a relative humidity of 45-70%.

Sampling commenced fifteen days after transplanting. Five samples of ten shoots from each treatment were taken at intervals of five days. Samples were oven dried (at 60˚C) to a constant weight and finely ground for bioassay. Chlorophyll was extracted with 80% acetone, and absorbed spectrophoto-metrically at 645 and 633 nm. Total amino acids and proline were determined as described by Moore and Stein (1954). Dry weights were expressed as g/ plant. All other parameters were determined as mg/g dry weight. Fresh samples were used for ascorbic acid determination (as mg/100 g fresh weight) which was made on the basis of the colorimetric method using 2,6 – dichloroindophenol (Holum and Denison 1982).

RESULTS AND DISCUSSION

Salt treatment of *Ercua sativa* seeds reduced their rate of germination from about 62% (Fig.1). But when salinized seeds were presoaked in either arginine or uric acid, the percent of seed germination increased to 80%. On the other hand, treatment of non – salinized seeds with either of the amino acids invoked no significant change in their rates of germination ($P > 0.05$).

Hence, it is assumed that exogenous amino acids improve seed tolerance to salinity significantly.

The total dry weights of salinized young plants were consistently significantly lower than those of non-salinized plants of the same sampling period ($p < 0.05$) (Fig. 2). For example, at the end of the experiment the dry weights of solely salinized plants were less than half those of the control plants, it may be attributed to deterioration in photosynthetic efficacy. Acid-treated salinized plants produced dry weights nearly similar to the control ones. Yet, acid treatment of non-salinized plants significantly increased their dry weights. As gauged by the dry weights, uric acid slightly surpassed arginine in improving growth of all plants.

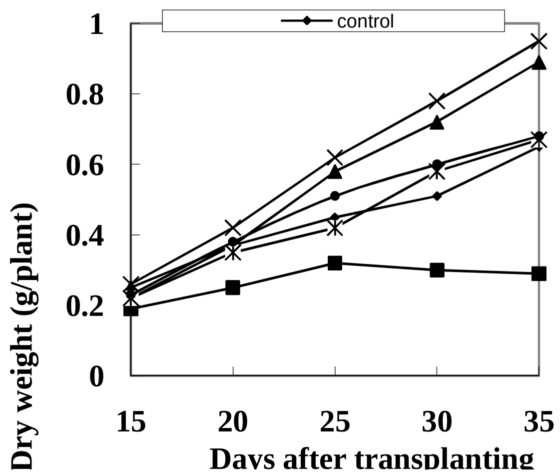

Fig. (1): Germination (%) in *Eruca Sativa* Seeds Treated with Amino Acids under Saline and Non-saline Conditions (c. control; s. NaCl; a. Arginine; u. Uric Acid).

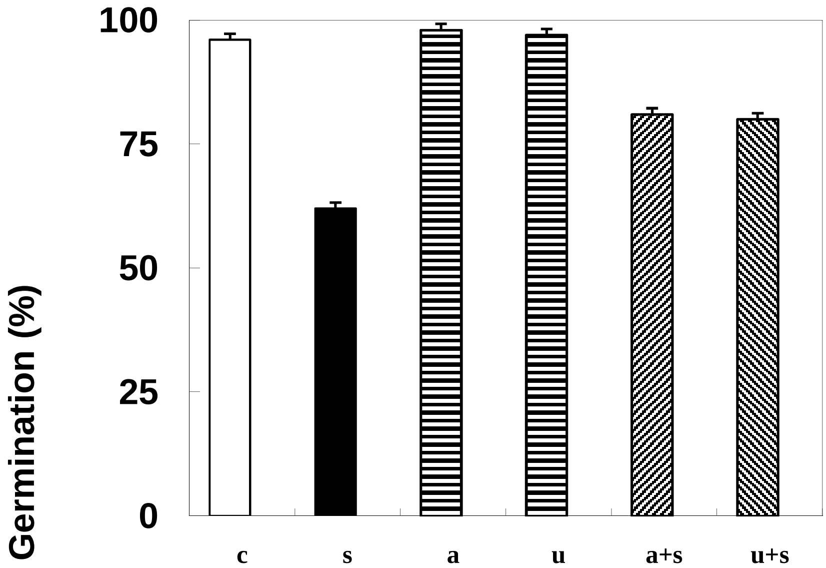

Fig. (2): Changes in Dry Weigh (g/plant) in *Fruca sativa* Shoots Treated with Amino Acids under Saline and Non-saline Condition

In all treatments, chlorophyll contents of *E. sativa* shoots increased over the duration of the experiment (Fig. 3). Depending on the treatment, they reached from about 1.5-fold (in solely salinized plants) to over 4-fold (in arginine and uric acid-treated non- salinized plants) the initial level. It is clear from the results that the use of exogenous acids counteracted the injurious effects of salinity and improved chlorophyll contents to levels considerably higher than those recorded in control plants. It is thus likely that these exogenous acids conferred an appreciable degree of salt tolerance and, hence, sustenance of chlorophyll synthesis systems in the plants. Yeo and Flowers (1986) found that the decrease in chlorophyll level in rice shoots was consistent with sodium ion increase. Zidan (1991) also reported that the use of certain vitamins had reduced the harmful effects of salinity on chlorophyll content of wheat plants.

The level of ascorbic acid in plants that received salt treatment only was consistently much lower than those of all other plants (Fig. 4). It showed little increase until day 25 when it decreased again to near its initial level. In all other treatments, it increased gradually with time to about 2–2.5-fold the initial level. The application of exogenous acids with NaCl stimulated increase of ascorbic acid to levels even greater than those of control plants, particularly from the middle of the experiment. Treatment with arginine or uric acid only had the greatest effect.

Total amino acids (Fig. 5) as well as the amino acid proline (Fig. 6) increased gradually over the experimental period in all treatments. The contents of total amino acids were higher in solely salinized plants than in plants that received both exogenous amino acids and NaCl simultaneously. Similarly their levels were significantly higher in salinized than non-salinized plants ($P < 0.05$), possibly due to protein degradation in response to Na^+ build-up. But the levels of proline were consistently highest in plants that had received the combined treatment of exogenous amino acids plus NaCl. Throughout the experimental period their levels were also significantly higher in salinized plants than in either amino acid- treated or control plants ($P < 0.5$). The results strongly suggest that these organic solutes promote salt tolerance in *E. sativa*. Accumulation of amino acids as well as proline was ascertained in different halophytes and non –halophytes under salinity conditions (Greenway and Munn 1980; Harborne 1988; Dubey and Rani 1989). It was suggested that build up of proline and other organic solutes in shoots of salinized plants either contributes to osmotic balance in cells (Stewart and Lee 1974; Zidan Yagi and S Al-Abdulkareem 1991) or helps to maintain enzymes activities (Pollard and Wyn Jones 1979; Greenway and Munns 1980).

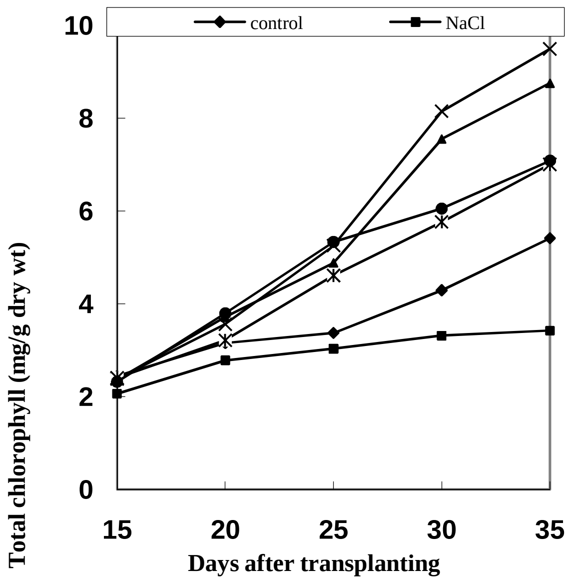

Fig. (3): Changes in Chlorophyll Content (mg/g dry weight) in Eruca sativa Shoots Treated with Amino Acids under Saline and Non-saline Conditions

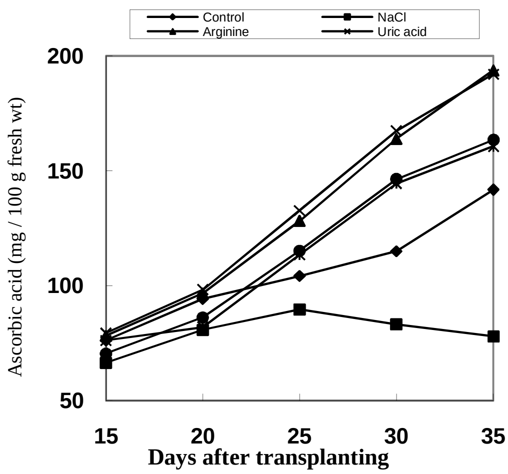

Fig. (4): Changes in Ascorbic Acid Content (mg/ 100 g Fresh Weight) in *Eruca sativa* Shoots Treated with Amino Acids under Saline and Non-saline Conditions

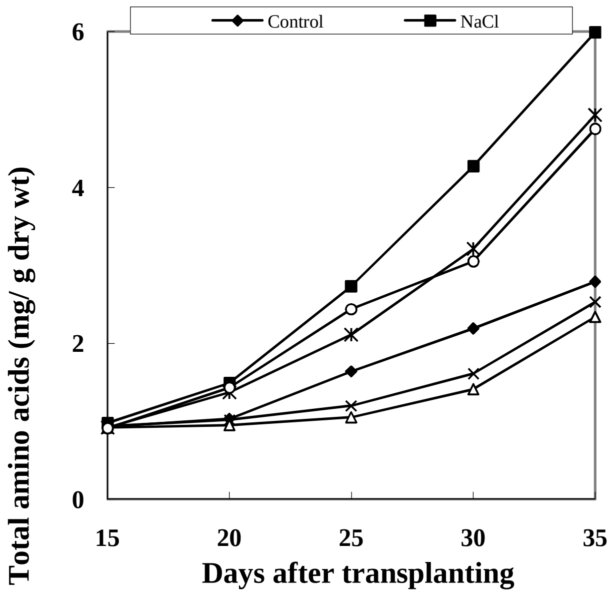

Fig. (5): Changes in Total Amino Acids (mg/g dryweight) in *Eruca sativa* Shoots Treated with Amino Acids under Saline and Non-saline Conditions

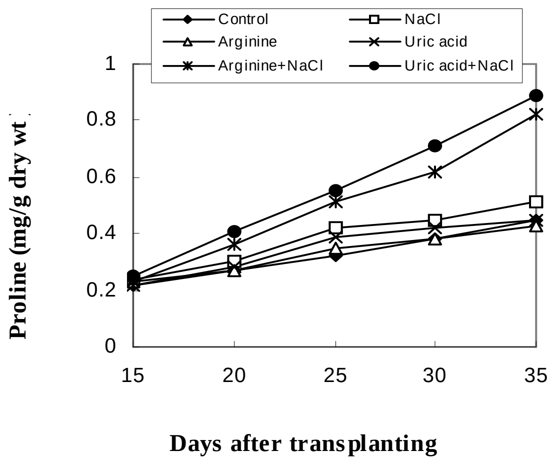

Fig. (6): Changes in Proline Content (mg/g Dry weight) in Eruca sativa Shoots Treated with Amino Acids under Saline and Non-saline Conditions

Our results indicate that treatment of *E. sativa* seeds and seedlings with either exogenous amino acids helps to improve their survival under salinity conditions. Since arginine and uric acid are precursors for polyamines, it is presumed that these acids might have helped in the synthesis of polyamines, which are associated with the adaptation to stressful environments (Kuehn *et al.*1990).

REFERENCE

1- **Aspinall, D.** (1986). Metabolic effects of water and salinity stress in relation to expansion of leaf surface. Aust J Plant Physiol **13:** [59-73].

2- **Balki, A. S. And Padole, V. R.** (1982). Effect of soaking seed treatments with plant hormones on wheat under conditions of soil salinity. J Indian Soc. Soil Sci. **30:** PP: [361-365].

3- **Basalah, M. O.** (1991). Effect of soaking on seed germination and growth of squash (*Cucurbita pepo L*) Seeding. Arab Gulf J Scient Res. **9:** PP: [87-97].

4- **Dubey, R. S. And Rani, M.** (1989). Influence of NaCI salinity on growth and metabolic status of protein amino acids in rice seeding. J. Agron and Crop Sci. **162:** PP: [97-106].

5- **Fallatah, O. And Hussai, G.** (1988). Effect of salt-water irrigation on germination, growth, and dry matter production of some faba beans (*Vicia faba*) cultivars. Proc. Saudi Biol Soc. **11:** PP: [11-18].

6- **Flowers, T. J. And Yeo, A. R.** (1986). Ion relations of plants under drought and salinity. Aust J Plant Physiol **13:** [75-91].

7- **Greenway, H. And Munns, R.** (1980). Mechanisms of salt tolerance in non-halophytes. Ann Rev. Plant Physiol **31:** PP: [149-190].

8- **Greenway, H. And Munns, R.** (1983). Interaction between growth, CI vacuolated cells. Plant Cell Environ **6:** PP: [567-574].

9- **Greenway, H. And Munns, R.** (1983). Interaction between growth, uptake of CI and Na and water relations of plants in saline environment. II. Highly vacuolated cells. Plant Cell Environ **6:** PP: [575-589].

10- **Hale, M. G. And Orcutt, D. M.** (1987).The Physiology of Plants Under Stress. John Wiley and Sons, Inc. USA, PP: [93-100].

11- **Harborne, J. B.** (1988). Introduction to Ecological Biochemistry. Academic Press, San Diego, USA PP: [26-31].
12- **Hoffman, G. J. And Ppene, C. J.** (1971). Effects of constant salinity levels on water use efficacy of bean and cotton. Trans Am. Soc. Agric Eng. **14:** PP: [1103-1106].
13- **Holum, J. R. and Denison, R. C**. (1982). Fundamentals of General, Organic and Biological Chemistry. John Wiley and Sons, NY PP: [338-339].
14- **Kuehn, G. D. Rodriguez-Garay, B. Bagga, S. And Phillips, G. C.** (1990). Novel occurrence of uncommon polyamines in higher plants. Plant Physiol **94:** PP: [855-857].
15- **Maas, E. V. And Nieman, R. H.** (1978). Physiology of Plant Tolerance to Salinity. In: Jung, G. A. *et al.* (ed) Am. Soc. Agron, Madison, Wi, PP: [277-299].
16- **McCre, K. J.** (1986). Whole-plant carbon balance during osmotic adjustment to drought and salinity stress. Aust. J. Plant Physiol **13:** [33-43].
17- **McCre, K. J. Kallsen, C. E. Richardson, S. G.** (1984). Carbon balance of sorghum plants during osmotic adjustment to water stress. J. Plant Physiol **76:** [898-902].
18- **Moore, K. and Stein, H. W**. (1954). A modifield ningydrin method for the photometric determination of amino acids and related compounds. J. Biol. Chem**. 211:** PP: [907-913].
19- **Munns, R. and Termaat, A.** (1986). Whole plant responses to salinity. Aust. J. Plant Physiol **13:** PP: [143-160].
20- **Pollard, A. and Wyn Jone, R. G.** (1979). Enzymes activities in concentrated solutions of glycinebtaine and other solutes. Planta **144:** PP: [291-298].
21- **Prakash, L. Dutt, M. and Prathapasenan, G.** (1988). Sodium chloride alters contents of nucleic acids, protein, polyamines and the activity of agmatine deiminas during germination and seedling growth of rice *Oryza sativa* L. Plant Physiol **15:** PP: [769-776].
22- **Rain, D. W.** (1972). Salt transport by plants in relation to salinity. Ann Rev. Plant Physiol **23:** PP: [367-388].
23- **Stewart, G. R. and Lee, J. A.** (1974). The role of praline accumulation in halophytes. Planta **120:** PP: [279-289].

24- **Yeo, A. R. and Flowers, T. J.** (1986). Salinity resistance in rice *Oryza sativa* and a pyramiding approach to breeding varieties for saline soils. Aust J. Plant Physiol **13:** PP: [161-173].

25- **Zidan, M. A.** (1991). Alleviation of salinity stress on growth and related parameters in wheat sprayed with thiamine, nicotinic acid, or pyridoxine. Arab Gulf J. Scient. Res. **9:** PP: [103-117].