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PARTIAL ALLEVIATION OF THE ADVERSE EFFECTS OF SALT STRESS ON *Zea mays* L. SEEDLINGS BY ACETYL SALICYLIC ACID (ASPIRIN).

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ABSTRACT

Sodium chloride salinity (60 mM) caused reduction of nitrates in the roots and leaves of*Zeamays* relative to control by 18% and 62% respectively. Foliar application of acetyl salicylic acid (5 mM) partially mitigated the adverse effects of salinity on the content of the ion. Nitrate reductase activity was also negatively affected by the salinity treatment and decreased by 81% and 29% in the roots and leaves respectively. Supplements of aspirin partially alleviated the activity and soluble sugars behaved similarly.

INTRODUCTION

Soil salinity is one of the major environmental factors limiting plant growth and productivity. It is estimated that one third of world's cultivated land is affected by salinity (Kaya *et al.,* 2002). Excess sodium chloride in the soil solution interferes with mineral nutrition and water uptake and ultimately lead to accumulation of toxic ions in crops.

Scanty information have revealed that salicylates can seriously interfere with metabolic processes during germination, seedling and later growth stages. Salicylic acid has been shown as an important signal molecule for modulating plant responses to environmental stress (Raskin, 1992). The ameliorative effects of salicylic acid have been recorded in inducing salt tolerance in tomato (Stevens et al., 2006). Wasfi (2014) has shown that foliar application of salicylic acid to *Phaseolus vulgaris* seedlings partially mitigated the adverse effects of salinity.

The objective of this work focuses on the effect of foliar application of acetyl salicylic acid (aspirin) on nitrate uptake and reduction and soluble sugars in seedling of *Zea mays* grown under high sodium chloride salinity.

MATERIALS AND METHODS

Plant Culture:

Selected grains of *Zea mays* obtained from the Faculty of Agriculture, University of Khartoum were surface sterilized for 10 minutes with 50% ethanol, rinsed several times with distilled water, and germinated in pots containing sand and soil (1/1), and watered every day with tap water. After three weeks the seedlings were divided into three sets as follows:

a) Set 1, controls.
b) Set 2, 60mMNaCl.
c) Set 3. 60 mMNaCl + 5 mM acetyl salicylic acid as foliar spray.

Three days later, roots and leaves were assayed for nitrate reductase activity, nitrate content and soluble sugars.

Nitrate assay:

Dried samples were ground, and the powder further dried at 70°C. One hundred mg of powder were mixed with 10 ml distilled water for 1 h at 45°C and then centrifuged (5000 rpm) for 5 min. The supernatant was used for nitrate determination by the salicylic acid method (Cataldo *et al.,* 1975). To 0.2 ml of the extract 0.8 ml of the salicylic acid reagent (5% in cone. H_2 SO_4) were added and the mixture left to cool. Then, 19 ml of 2N NaOH solution were added, and the nitrate content was measured spectrophotometrically at 410 nm, and concentrations were derived from a standard curve.

Assay of nitrate reductase:

In vivo nitrate reductase was assayed as outlined by Radin (1978). One g of fresh material was thoroughly washed and incubated for 1 h in 10 ml potassium phosphate buffer (pH 7.5) containing few drops of 1% l-propanol as wetting agent. Prior to assay, the buffer solution was purged with N_2gas for 30 min to remove oxygen. Nitrite was quantitatively released into the medium, and it was determined by combining 1 ml dilute sample with 1 ml sulfanilamide (1% w/v in 1.5M HC1), and 1 ml naphthylethlenediamine hydrochloride (0.02% w/v). After 15 min, absorbance was read at 540 nm in a spectrophotometer and nitrite concentrations (representing nitrate reductase activity) were calculated from a standard curve.

Determination of soluble sugars:

Soluble sugars were extracted by grinding 0.5g of fresh material in a pestle and mortar with 80% ethanol. The extract was filtered through Whatman filter paper No. 1. The filtrate was heated in a water bath to evaporate the ethanol, and the remaining residue wasmade to 10 ml by distilled water.

The soluble sugars were assayed using the anthrone method asdescribed by Halhoul and Kleinberg(1972). Six ml of anthrone reagent (0.15% in cone. H_2S0_4) were added to 0.4 ml distilled water and 0.1 ml of the sugar extract in a test tube placed in a boiling water bath for 6 min. afterwards, the tube was transferred to cold water to stop the reaction. Absorbance was read at 630 nm in a spectrophotometer and calculations were made from a standard curve.

RESULTS AND DISCUSSION

Results represented in Fig. (1)show the effect of salinity (60 mM) on the nitrate content in the roots and leaves of *Zea mays* seedlings. Salinity caused reduction of the nitrate content by 18% and 62% in the roots and leaves respectively. An obvious ameliorative effect was observed when acetyl salicylic acid (5mM) was foliar applied to salinized seedlings (Fig. 1).

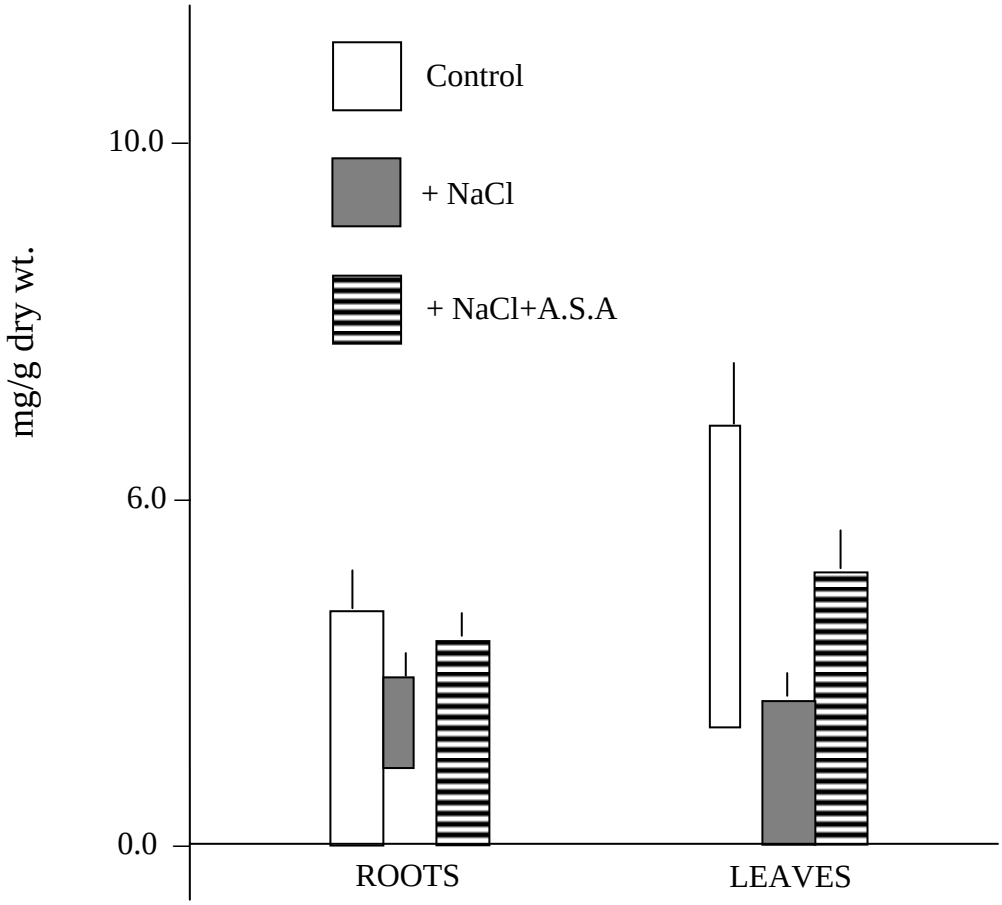

Fig. 1.: Effect of salinity and acetyl salicylic acid (A.S.A) addition on nitrate content in the roots and leaves of *Zea mays*seedlings. Error bars represent ± standard deviations of three replicates.

Nitrate reductase activity (Fig. 2) also showed distinct decline as a result of salinity treatment, and reduction of the enzyme relative to controls amounted to 81% in the roots and 29% in the leaves. Again, foliar application of acetyl salicylic acid partially alleviated the depressive effect of salinity on the enzyme activity.

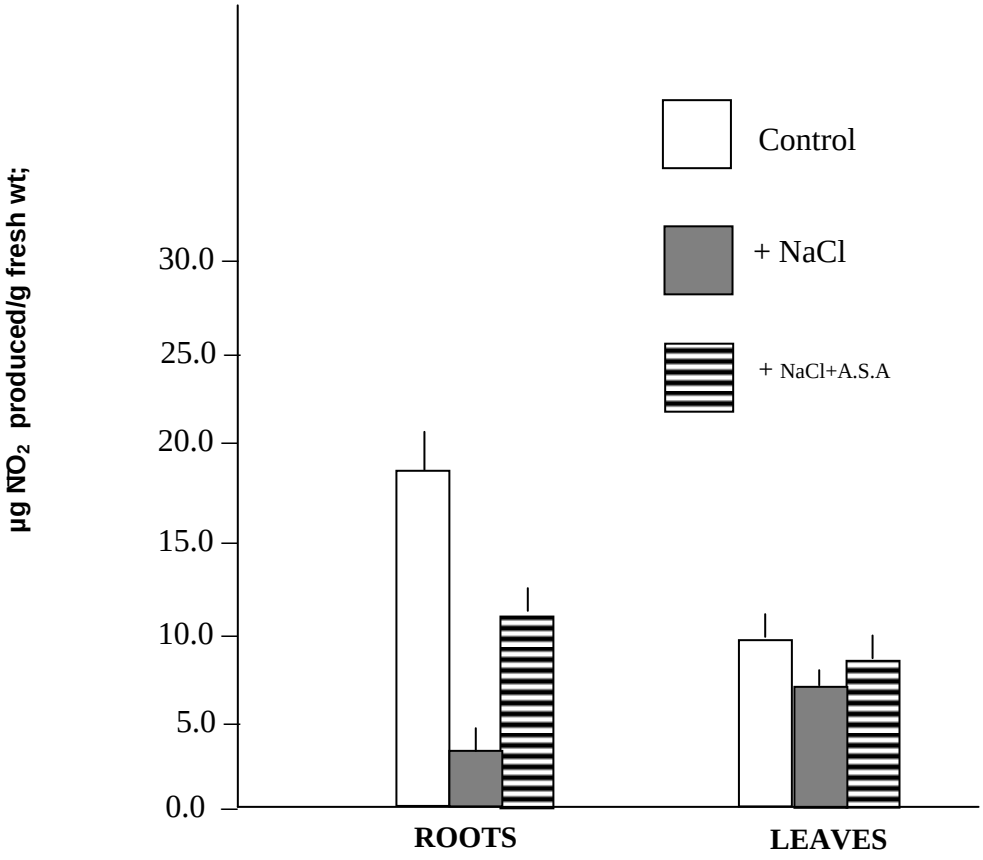

Fig. 2.: Effect of salinity and acetyl salicylic acid (A.S.A) addition on nitrate reductaseactivity in the roots and leaves of *Zea mays*seedlings. Error bars represent ± standard deviations of three replicates.

Soluble sugars showed a similar trend with distinct reduction in the salinized treatments and obvious elevation of the parameter with acetyl salicylic acid application.

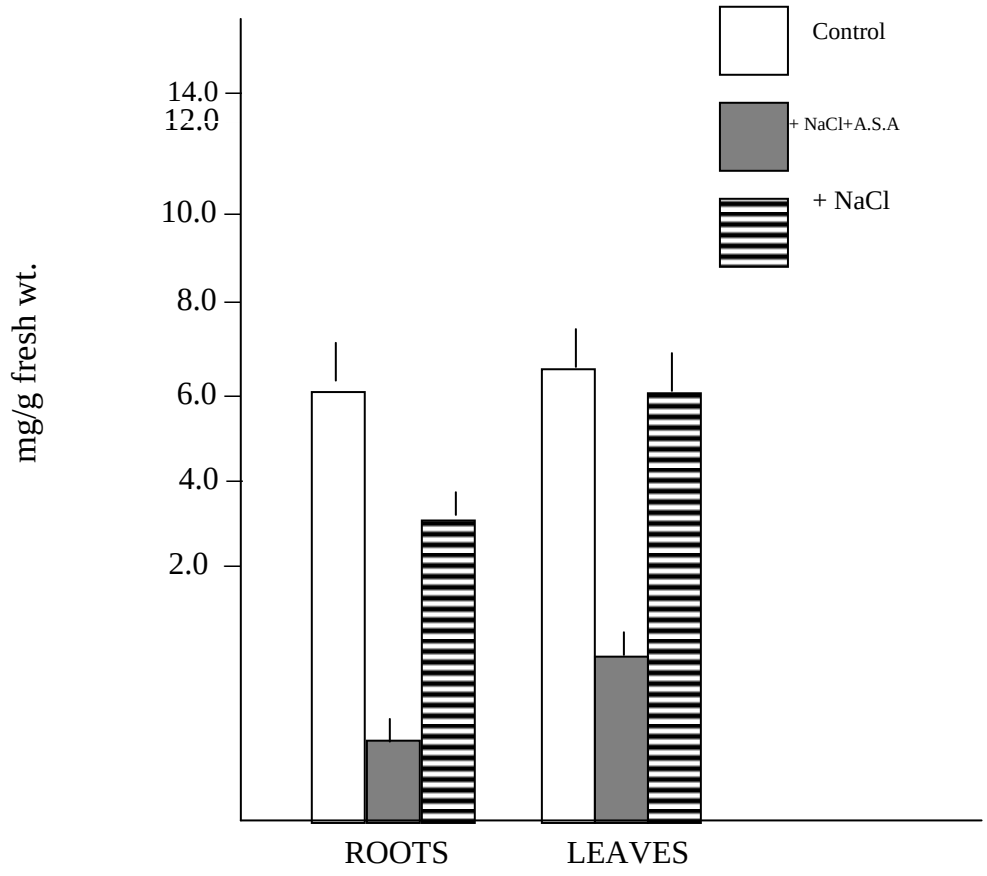

Fig. 3.: Effect of salinity and acetyl salicylic acid (A.S.A) supplement on soluble sugars in the roots and leaves of *Zea mays*seedlings. Error bars represent ± standard deviations of three replicates.

Nitrates and soluble sugars are vital and closely related with nitrate reductase, since nitrates are the substrates for de novo synthesis of the enzyme, and soluble sugars provide the driving force and energy needed for nitrate uptake, translocation and ultimately reduction.

The results obtained in this study are consistent with those of Khan *et al.,* (2003) who found that spraying low concentrations of salicylic acid and acetyl salicylic acid on the leaves of soybean and corn led to an increase in the overall photosynthetic yield. Ferrududdin*et al.,* (2003) observed an increase in the nitrate reductase activity, net photosynthetic rate, carboxylation efficiency and seed yield due to supplements of salicylic acid in *Brassica juncea.* Other reports have shown that salicylates can positively encounter the deleterious effects of plants grown under salt stress. Salicylic acid controls salinity tolerance in wheat (Shakirova and Bezrukova, 1997 and Singh and Usha,2003), mineral nutrition and oxidative stress in maize (Gunes*et al.,* 2007). Stevens, *et al.,* (2006) have shown that acetyl salicylic acid and salicylic acid induce multiple stress tolerance in bean and tomato plants.

These results and reports on the physiological and biochemical effects caused by salicylates suggest that these substances might positively interfere in growth regulation of plants, but further studies are required in order to determine their efficiency under natural field conditions.

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تخفيف التاثيرات المعاكسة للاجهاد الملحى على بادرات الذرة الشامية بواسطة اسيتيل حامض السليسيليك(اسبرين)

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ادت ملوحة كلوريد الصوديوم(٦٠مليمول)الى اختزال النترات فى جذور واوراق الذرة الشامية مقارنة بالكنترول بنسبة ١٨%و٦٢% على التوالى. كما ادت المعاملة رشا باسيتيل حامض السليسيليك (٥مليمول)الى تخفيف جزئى للتاثيرات المعاكسة للملوحة على المحتوى الايونى. كما تاثر سلبيا ايضا النشاط الانزيمى لاختزال النترات بمعاملة الملوحة ونقص بنسبة ٨١%و٢٩% فى كل من الجذور والاوراق على التوالى. اضافة الاسبرين ادت الى التغلب جزئيا على هذا النشاط كما ان السكريات الذائبة تاثرت بطريقة مشابهة.