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Effect of Salicylates on Nitrate Content and Reduction in Salt – Stressed *Zea mays* L. Seedlings

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ABSTRACT

This study was conducted to evaluate the leaf response of maize to high salt – stress conditions and supplements of salicylates. Sodium chloride salinity (50mM) distinctly diminished the nitrate content, and nitrate *reductase*, being a substrate induced enzyme behaved similarly. Supplements of salicylates (salicylic acid, acetyl salicylic acid and methyl salicylates partially alleviated the depressive effects of salinity on the two assayed parameters.

INTRODUCTION

Salinity is one of the limiting environmental factors for soil fertility and plant production (Khan and Panda, 2008). It is estimated that about one third of world's cultivated land is affected by salinity (Kaya et al., 2002). Basic cause of salinity–induced effects on growth and development of plants is accumulation of ions in soil solution and ultimately in plant cells. These ions rise to toxic levels and impose an additional stress on physiological and biochemical processes. Therefore, the development of methods and strategies to ameliorate deleterious effects of salt stress on plants has received considerable attention in the past few years.

Salicylic acid which is a secondary plant product plays vital roles in the growth of plants. It is a potent signaling molecule in plants and is involved in inducing response to biotic and abiotic stresses (Kaur et al., 2009). Ray et al., (1986) claimed that salicylic acid and similar phenolic compounds provide resistance against different stress factors. These observations on the ameliorative effect of salicylic acid in plants growing under severe stress conditions might qualify salicylic acid as a new plant growth regulator (Hayat and Ahmed, 2007).

The objective of this work focuses on the effect of foliar application of salicylic acid and its derivatives on nitrate uptake and reduction in *Zea mays* seedlings grown under salt stress.

MATERIALS AND METHODS

Plant culture:

Grains of *Zea mays* L.var. Mugtama 45 obtained from the Faculty of Agriculture, University of Khartoum were first disinfected with 0.5% sodium hypochlorite solution for 5 min. and then washed several times with distilled water and germinated in pots under greenhouse conditions. The pots contained sand and clay (1:1), and watered daily. After three weeks, the pots were divided into 5 sets as follows:

1- Controls.
2- Salinized with 50mM NaCl.
3- Salinized with 50mM NaCl + 5mM Salicylic Acid (ASA).
4- Salinized with 50mM NaCl + 5mM Methyl Salicylate (MS).

The salinity solutions (50mM NaCl) were irrigated in the rooting medium, whereas the salicylates were foliar applied. Five days later, leaves of all treatments were assayed for nitrate content and nitrate *reductase* activity.

Nitrate assay:

Dried samples were ground, and the powder further dried at 70ºC. One hundred mg of powder were mixed with 10ml distilled water for 1 h at 45ºC, 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 0.2ml of extract 0.8ml of the salicylic acid reagent (5% in conc. H_2SO_4) were added and the mixture left to cool. Then,19ml of 2N NaOH solution were added, and the nitrate content was measured spectrophotometrically at 410nm, 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 10ml potassium phosphate buffer (pH 7.5) containing few drops of 1% 1- propanol as wetting agent. Prior to assay, the buffer solution was purged with N_2 gas for 30min to remove oxygen. Nitrite was quantitatively released into the medium, and it was determined by combining 1ml dilute sample with 1ml sulfanilamide (1% in 1.5 M HCl) and 1ml naphthylethlene diamine hydrochloride (0.02%). After 15 min, absorbance was read at 540nm in a spectrophotometer, and nitrite concentrations (representing nitrate *reductase* activity) were calculated from a standard curve.

RESULTS AND DISCUSSION

Nitrate content (Fig. 1) and nitrate *reductase*activity (Fig. 2) were obviously diminished by salinity treatments. Foliar application of salicylic acid and its derivatives partially alleviated the depressive effects of salinity on the two parameters. These findings are consistent with Fariduddin et al., (2003) who observed increase of nitrate*reductase* activity, net photosynthetic rate, carboxylation efficiency and seed yield due to low concentrations of salicylic acid in *Brassica juncea*. Khan et al., (2003) found that spraying low concentrations of salicylic acid and acetyl salicylic acid on the leaves led to an increase in the overall photosynthetic yield of soybean and corn.

Many other reports have shown that salicylic acid and its derivatives can positively encounter the deleterious effects of plants grown under salt stress. Salicylic acid controls salinity tolerance in wheat (Shakirova and Benzrakova, 1997), osmotic stress in wheat (Singh and Usha, 2003), mineral nutrition and oxidative stress in maize (Gunes et al., 2007), nitrate uptake and reduction in *Phaseolus vulgaris* (Wasfi, 2014).

Salicylic acid which belongs to a group of plant phenolics is widely distributed in plants, and is now considered as a hormone – like substance which plays an important role in the regulation of plant growth and development (Raskin, 1992). Salicylic acid has been reputed to cause a multitude of effects on the morphology and physiology of plants (Pancheva et al., 1996), and to induce a protective mechanism enhancing resistance to biotic and abiotic stresses (Lopez-Delgado et al., 1998).

Results obtained in this study showed that salicylic acid and its derivatives could protect *Zea mays* seedlings from the detrimental effects of salt stress by improving the two tested key parameters.

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تأثير الساليسيلات على محتوي واختزال النترات في بادرات الذرة الشامية الناميه تحت ظروف الملوحة

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أجريت هذه الدراسة لتقييم استجابة أوراق الذرة الشامية الناميه تحت ظروف ملوحة عالية وتأثير رش الساليسيلات على المجموع الخضري. كلوريد الصوديوم بتركيز ٥٠ مليمولر أحدث انخفاضاً واضحاً في محتوي النترات، وأنزيم النترات روكينز الذي يحفز تخليقه بواسطة النترات انخفض كذلك. إضافة الساليسيلات (حمض الساليسليك واستيل حمص الساليليك وساليسيلات الميثيل خفضت والغت جزئياً الأثر الضار للملوحة على المتغيرين.