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TWO PHENOLIC ACIDS AMELIORATE THE ADVERSE EFFECTS OF SALT STRESS ON *Zea mays* L. SEEDLINGS.

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ABSTRACT

Pot experiments were conducted to evaluate the effect of foliar application of salicylic acid (3 mM) and benzoic acid (3 mM) on nitrate content and nitrate reductase activity in leaves of *Zea mays* seedlings grown under high salt stress (50 mM NaCl). Salinity treatment caused diminishment of the nitrate content and nitrate reductase activity by approximately 78% and 65% respectively.

Supplements of salicylic acid and benzoic acid partially alleviated the depressive effect of salinity on the two parameters, but did not completely recover to control values. Salicylic acid supplement proved to be more effective in encountering the deleterious effects of salinity.

INTRODUCTION

Soil 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' 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 the deleterious effects of salt stress on plants has received considerable attention in the past few years. The agricultural problem of salinity tolerance is probably tackled by either altering farming practices to prevent soil salinization occurring in the first place, or by implementing schemes to try to remediate salinized soil (such as by planting perennials to lower water tables), or by certain biological and chemical treatments such as supplements of potassium (Massaad, 2010) or addition of certain phenotic acids (Wasfi, 2014).

Plant phenolics have often been referred to as secondary metabolites. The term "secondary" implied that such compounds were only of minor importance to the plant and could sometimes be equated with waste products (Raskin, 1992). It is now well documented that many phenolic compounds play an essential role in the regulation of plant growth, development and interaction with other organisms (Harbone, 1980).

The objective of this work focuses on the effect of foliar application of salicylic acid (3 mM) and benzoic acid (3 mM) on nitrate uptake and reduction in leaves of *Zea mays* seedlings grown under high salt stress (50 mM NaCl).

MATERIALS AND METHODS

Plant culture:

Grains of *Zea mays* var. Mugtam were surface sterilized for 5 min in 50% alcohol, and then washed several times with distilled water, and germinated in pots containing sand and clay (1:1), and watered every other day. Seedlings of comparable size aged -three weeks were used in this study. The two phenolic acids were foliary applied using suitable sprayers, and great care was taken to prevent the phenolic acids from coming into direct contact with the soil. Nitrate content and nitrate reductase activity were assayed 5 days after treatments.

Nitrate assay:

Dried samples were ground, and the powder further dried at 70ºC. One hundred mg of powder were mixed with 10 ml 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 0.2 ml of the extract 0.8 ml of the salicylic acid reagent (5% in conc. 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.

Nitrate reductase assay:

In vivo nitrate reductase was assayed as outlined by Radin (1978). One g of leaf discs was thoroughly washed and incubated for 1 h in 10 ml potassium buffer (pH 7.5) containing few drops of 1% 1−propanol as wetting agent. Nitrite is quantitatively released into the medium, and it was determined by combining 1 ml dilute sample with 1 ml sulfanilamide (1% in 1.5 M HCl), and 1 ml naphthylethlene diamine hydrochloride (0.02% in water). After 15 min, absorbance was read at 540 nm and nitrite concentrations (representing nitrate reductase activity) were calculated from a standard curve.

Results and Discussion

Fig. 1 shows the effect of salt stress and two phenolic acids on nitrate content in the leaves of *Zea mays* seedlings grown under high salt stress. It is evident that the ion content was distinctly affected by salinity and phenolic acids treatments. Salt stress decreased the ion content by approximately 78% as compared to non saline treatment. Salicylic acid and benzoic acid complements partially mitigated the adverse effect of salinity as shown in Fig. 1.

Fig. l: Effect of NaCl salinity (50 mM), salicylic acid (S.A, 3mM) and benzoic acid (B.A, 3 mM) on nitrate content in leaves of *Zea mays* seedlings. Error bars represent ± standard deviations of three replicates.

Nitrate reductase, being a substrate dependent enzyme for de novo synthesis followed a similar trend with vivid reduction of the enzyme activity by salinity treatment relative to controls (65% reduction). Addition of the two phenolic acids partially alleviated the deleterious effects of salinity (Fig. 2).

Fig. 2: Effect of NaCl salinity (50 mM), salicylic acid (S.A, 3mM) and benzoic acid (B.A, 3 mM) on nitrate reductase activity in leaves of *Zea mays* seedlings. Error bars represent ± standard deviations of three replicates.

These results are consistent with Wasfi (2014) who showed that salicylic acid partially alleviated the adverse effects of salinity on *Phaseolous vulgaris*. Khan *et al*., (2003) found that spraying low concentrations of salicylic acid on leaves of soybean and corn led to an increase in the overall photosynthetic yield. Results obtained in this study are further substantiated by Fariduddin *et al*., (2003) who observed an increase in nitrate reductase

activity, net photosynthetic rate, carboxylation efficiency and seed yield due to low doses of salicylic acid in *Brassica juncea.* Yildirim *et al*., (2008) found that exogenous salicylic acid application inhibited Na accumulation, but stimulated N, P, K, Mg, Fe, Mn and Ca uptake. An increase in concentration of K and Ca in plants under salt stress could ameliorate the deleterious effects of salinity on growth and yield (Grattan and Grieve, 1999).

Scanty information is available concerning the effect of benzoic acid on plants grown under high salt stress. Watanabe *et al*., (1981) and Fujoka *et al*., (1983) have reported ameliorative effects of benzoic acid and its derivatives on the regulation and growth of some *Lemna* spp. especially during the flowering stage.

It is worthmentioning that the two phenolic acids used in this study did not completely recover the adverse effects of salinity, but both of them partially alleviated the negative effects of salinity on the two parameters. Salicylic acid outclassed benzoic acid in encountering the deleterious effects of salinity.

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خفض الاثار الضارة للملوحة على بادرات الذرة الشامية باستخدام حامضين فينوليين

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أجريت تجارب أصص لدراسة تأثير رش حمضي السالسليك والبنزويك بتركيز ٣ مليمولر لكل منهما على محتوي النترات وإنزيم النترات ردكتيز في أوراق بادرات الذرة الشامية النامية تحت تركيز عالي من الملوحة (٥٠ مليمولر كلوريد الصوديوم).

البادرات التي تعرضت للملوحة أنخفض فيها محتوي النترات وتركيز إنزيم النترات ردكتيز بـ ٧٨% و ٦٥% على التوالى مقارنة مع بادرات المقارنة.

عند رش الحمضين نتج تحسن وإلغاء جزئي للأثر السلبي للملوحة على المتغيرين ولكن لم يتم استعادة التركيزيين كما في بادرات المقارنة. حمض السالسليك كان أكثر كفاءة من حمض البنزويك في التغلب على الأثر السلبي للملوحة.

قام بتحكيم البحث

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