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EFFECTS OF SALINITY AND ZINC ON PROFILIN GENE (PRF1) EXPRESSION, GROWTH AND PEROXIDASE ACTIVITY OF WATERMELON PLANT.

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ABSTRACT

As sessile plants have developed adaptive strategies to cope with environmental stress. The understanding of plants responses to their external environment is of importance with respect to basic research. It is also an attractive target for improving the performance of crop plants under stress conditions. This investigation showed that PRF1 expression in watermelon calli exposed to salinity was reduced, in contrast, the PRF1 expression was increased by zinc. Calli fresh weight exposed to 150 mM NaCl and 800 μM $ZnSO_4 . 7H_2O$ were decreased by 69% and 76%, respectively. Peroxidase activity was increased in salinity and zinc stressed calli. At 150 mM NaCl-stressed calli had a peroxidase activity 2.3 times higher than unstressed calli.
Keywords: Profilin gene, salinity, zinc, peroxidase, western blot, watermelon.

INTRODUCTION

Environmental stresses are among the factors most limiting to plant productivity. Such stresses are becoming even more prevalent as the intensity of agriculture practices increases. Therefore, elucidation of the mechanisms by which plants perceive and transduce these stresses is critical if we are to understand the plant response and introduce genetic or environmental improvement to stress tolerance (Borsani *et al.,* 2001). In Egypt, water resources are limited for agriculture expansion, hence our attention is directed to other resources than Nile water. It is believed that the increased usage of high salty waters for supplemental irrigation has created a need for a better understanding of the effect of salinity on crop production (El-Etreiby, 2002). Zinc content in soil samples collected from highly polluted agricultural soils exposed to prolonged irrigation with industrial wastewater in Egypt, was 410.3 ppm (Abdel-Sabour and Abdel-Basset, 2002).

In Egypt the first recorded watermelon harvest occurred nearly 5000 years ago in. Now Egypt currently ranks fifth worldwide in production of watermelon, by 38.151 $x10^6$ cwt in the year 2001 (National Agricultural Statistics Service, 2002). The actin-based cytoskeletal system of eukaryotic cells is complex, containing more than 70 distinct families of actin-binding proteins. The actin cytoskeleton contributes to many of the dynamic processes directing plant development (Kandasamy *et al.,* 2002). Because the actin binding protein profilin is particularly important to the dynamics of actin polymerization and sequestration, the regulation of plant profilin expression responding to salinity and zinc should affect plant development. In order to analyse the responses of plants to salinity and zinc at the molecular

level, Western blot was applied to unravel the level of PRF1 expression, in addition, calli growth rates and peroxidase activity were investigated.

MATERIALS AND METHODS

Culture Conditions

Culture of watermelon was conducted as previously described (Compton and Gray, 1993). Seeds of 'Crimson Sweet' variety were surface-disinfected for 30 min in 2.5% Na Clo plus one drop Triton X-100, rinsed five times with sterile-distilled water, and soaked overnight in sterile-distilled water in darkness. Embryos were extracted and were germinated on MS medium supplemented with (per liter) 20g sucrose, 100 mg myo-inositol, 2 mg glycine, 0.5 mg each of pyridoxine HCl and nicotinic acid, and 0.1 mg thiamine HCl. The pH of all media was adjusted to 5.7 before the addition of 7 g phytoagar/ liter and autoclaving. Explants consisted of cotyledons from 5-day-old seedlings excised and cultured on MS medium as above but with 30 g sucrose/liter, BA (20 μ M) and IAA (3 μM) for 6 weeks under a 16 h photoperiod at 25ºC. The effect of NaCl or $ZnSO_4$. $7H_2O$ at 0, 50, 100 and 150 mM or 0, 200, 400 and 800 μM, respectively, was examined by incubating the callus for 18 days on the supplemented medium.

Growth Measurements

Inhibition of growth due to NaCl or $ZnSO_4$. $7H_2O$ was scored by measuring fresh weight gain. The initial callus samples were weighed, and also after plated on medium containing the various concentrations of the cations. The growth coefficient was calculated as described by Frank *et al.* (2000).

Western blot analysis

Proteins from calli were extracted and analyzed by Western blotting as performed by Kandasamy *et al.* (2002).

Peroxidase assay

Parallel experiment was run to assay peroxidase activity. The calli were ground in 0.1 M phosphate buffer (pH 6) (2 ml/g fresh weight) in the presence of activated charcoal (50 mg/g fresh weight). After centrifugation, the supernatant solutions were dialyzed against 0.01 M sodium phosphate buffer (pH 6) overnight and used directly as a source of enzyme. Peroxidase activity was assayed in 0.02 M guaiacol, 0.1 M sodium phosphate buffer (pH 6), and 0.03 M H_2O_2. The increase in absorbance was monitored at 420 nm in a Beckman DU-50 spectrophotometer. Enzyme activity is expressed as units per mg protein, where one unit is defined as the amount of enzyme causing a decrease in absorbance of 1 per s for peroxidase (Negrel *et al.,* 1993). Protein content was determined by Bradford method (Bradford, 1976) using a BioRad protein assay kit.

RESULTS

The expression of PRF1 gene

Western blot was used to identify whether the expression of PRF1 was influenced by salinity and zinc or not. The results of Western blot showed

that the PRF1 expression in calli exposed to salinity was reduced. MAbPRF1a detected a strong, moderate and weak band at 50, 100 and 150 mM NaCl, respectively, compared with that at 0 mM NaCl (Fig 1A). In contrast, the PRF1 expression in calli exposed to zinc was increased compared with the non- treated calli (Fig 1B). The expression of PRF1 was correlated with zinc concentration (200, 400 & 800 μ M).

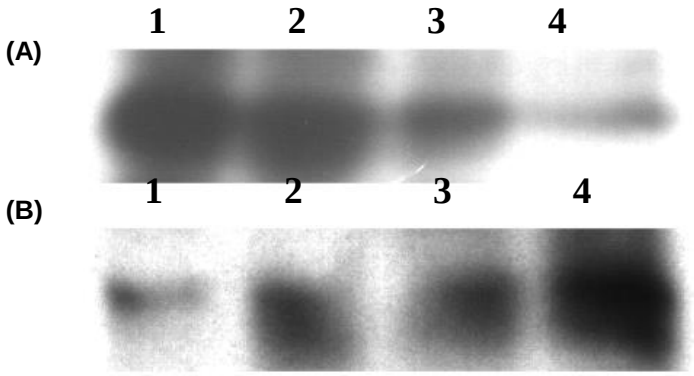

Fig.1. Immunoblot analysis of PRF1 expression. MAbPRF1 was used to probe protein extracts from watermelon calli exposed to (A) 0, 50, 100 & 150 mM NaCl in lanes 1, 2, 3 & 4, respectively. (B) 0, 200, 400 & 800 μM $ZnSO_4 . 7H_2O$ in lanes 1, 2, 3 & 4, respectively.

Calli growth

The effect of salinity and zinc on the growth of watermelon callus was measured. The results in Figure 2 show that the gain in fresh weight was for all treatments slower than for untreated callus. The growth coefficient of calli exposed to 100 and 150 mM NaCl was 0.55 and 0.46 respectively. Growth rate of callus exposed to 200 μM $ZnSO_4 . 7H_2O$ was decreased to half, compare to unexposed callus. Calli fresh weight exposed to 150 mM NaCl and 800 μM $ZnSO_4 . 7H_2O$ were decreased by 69 and 76%, respectively. These observations suggest that watermelon calli are more tolerant to salinity than zinc.

Determination of peroxidase

All stress treatments increased peroxidas activity in calli (Fig 3). Significant increases of peroxidase activity were already observed in salinity stressed calli. At 50, 100 & 150 mM NaCl-stressed calli had a peroxidase activity 1.3, 1.9 & 2.3 times more than control, respectively, while calli exposed to 200, 400 & 800 μ M $ZnSO_4 . 7H_2O$ had 1.2, 1.3 & 1.6 times, respectively, more peroxidase activity than unstressed calli.

fig2

fig3

DISCUSSION

Profilins, are small proteins involved in the regulation of the actin cytoskeleton. Besides being actin-binding proteins, profilins also bind poly-L-proline, formin homology domain-containing proteins, Arp 2/3 complex, and annexins. Available evidence suggests that profilin is a multifunctional protein that exerts positive and negative regulatory effects on actin polymerization and it may be involved in signal transduction (Ramachandran *et al.,* 2000).

The Western blot analysis revealed reduction of PRF1 expression by salinity. Gong *et al.* (2001) reported that AD05 C10/circadian rhythm-RNA bindingl (CCR1) gene was reduced in Arabidopsis plants after NaCl treatment. Actin relocalization was inhibited during osmotic stress (Rupes *et al.,* 1999). The data showed increment of PRF1 expression by zinc. Efremova *et al*. (2002) found that zinc cause a strong induction of the heat shock protein Hsp 70. Zinc exerted strong effects on the cytoskeleton (Schmuck *et al.,* 2002).

The data showed that at 150 mM NaCl, watermelon callus fresh weight was decreased by 69%. In accordance with this results, Garratt *et al*. (2002) reported that at 200 mM NaCl, cotton callus fresh weight was decreased by 52% (Tol) and 89% (MED). On the other hand, at 800 μM $ZnSO_4$. $7H_2O$, watermelon callus fresh weight was decreased by 76%. Maroti and Bognar (1988) demonstrated that the increase in fresh weight of tobacco and rue calli were inhibited by zinc sulfate to 75-87%.

The results of peroxidase, however, indicated that peroxidase activity was increased when watermelon calli treated with salinity and zinc. Reports from studies with treatment of melon and ryegrass by salinity and zinc showed increased levels of peroxidase (Bonnet *et al.,* 2000; Rodriguez - Lopez *et al.,* 2000).

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تـأثيرات الملوحـة والزنـك علـى تعبيـر جـين البـروفلين (PRF1)، والنمـو ونشـاط البيروكسيديز لنبات البطيخ

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النباتات أن تتوائم مع الاجهاد البيئى. تفهم استجابة النباتات لبيئتها الخارجية يعتبر ذو أهمية علمية وكذلك يعتبر هدف لتحسين الانتاج النباتى تحت ظروف الاجهاد. هذا البحث بين أن تعبير جين PRF1 فى كالوس البطيخ المعامل بالملوحة قد انخفض بينما زاد تعبير نفس الجين عند المعاملة بالزنك فأدت المعاملة إلى نقص الوزن الغص للكالوس المعامل بتركيز ١٥٠ مليمول كلوريد صوديوم و٨٠٠ ميكرومول كبريتات زنك بنسبة ٦٩ و٧٦% على الترتيب. وقد زاد نشاط البيروكسيديز فى الكالوس المعامل بالملوحة والزنك. فالكالوس المعامل بتركيز ١٥٠ مليمول كلوريد صوديوم و٨٠٠ ميكرومول كبريتات زنك اظهر زيادة فى نشاط البيروكسيديز ٢,٣ مرة و١,٦ مرة قدر الكالوس الغير معامل، على الترتيب.