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EFFECT OF GROWTH REGULATORS, SALINITY AND HEAVY METAL STRESSES ON ACID PHOSPHATASE ACTIVITY OF SUNFLOWER COTYLEDONS

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ABSTRACT

Acid phosphatase (EC 3. 1. 3. 2) activity was measured in sunflower (*Helianthus annus* L.) cotyledons. The present results indicate that the tested growth regulators indoleacetic acid (IAA), naphthylacetic acid (NAA) and indolephenoxyacetic acid (IPA) induced acid phosphatase activity at concentration range 100-400 µM. The enzyme was inhibited by $CdCl_2$ and $PbCl_2$ at 20-100 µM. Cd^{2+} and Pb^{2+} enhanced lipid peroxidation and H_2O_2 content. Malondialdehyde (MDA) content was reduced in cotyledons pretreated with GA_3. Salinity induced lipid peroxidation and increased H_2O_2 content. NaCl at low concentrations (50 and 100 mM) induced the enzyme activity whereas the higher concentrations (150-300 mM) reduced the activity. Coronatine (COR) at 100 µM alleviated partially the toxic effect of salinity.

INTRODUCTION

Acid phosphatases produced by plants are presumed to convert organic phosphorus into available Pi, which is absorbed by plants (Aoyama *et al.,* 2001). The hydrolysis process is linked to energy metabolism, metabolic regulation and a wide variety of cellular signal transduction pathways of plant cells (Vincent *et al.,* 1992).

Acid phosphatase was reported in plants e.g. soybean (Dewald *et al.*, 1992), *Lupinus albus* (Li *et al*., 2002), *Vigna sinensis* (Tapan *et al*., 1996), clover (Dracup *et al*., 1984), duckweed (Nakazato *et al.,* 1998) and tomato (Bozzo *et al.,* 2002).

A wide range of plants and plant tissues show acid phosphatase activity with substantial difference in protein size, tissue localization and regulation of expression (Duff *et al.,* 1994).

Acid phosphatase acts on different substrates such as: *p*-nitrophenylphosphate (Lee, 2000), α-naphthylphosphate (Saeed *et al*., 1998), pyrophosphate (Tapan *et al*., 1996) and α-glycerophosphate (Tso and Chen, 1997).

Acid phosphatases usually present in multiple forms, which differ in molecular mass, phosphate value, substrate specificity and electrophoretic mobility. In general, they occur in very small quantities in plant tissues, are unstable in diluted solutions, and are subject to surface denaturation in the pure state. Acid phosphatases display different biochemical properties and differ in their optimum pH, optimum temperature and metal ion requirements (Duff *et al.,* 1994).

Therefore, the aim of the present investigation was to measure the activity of acid phosphatase in cotyledons of *Helianthus annus* and investigating the effect of some abiotic factors such as salinity and heavy metal stresses.

MATERIALS AND METHODS

The experimental plant used in this investigation was *Helianthus annus* L, (sunflower, family: Compositae). Pure strain of seeds was obtained from Egyptian Ministry of Agriculture. Sunflower seeds were germinated according to El-Shora (2001) and acid phosphatase assay was carried out according to El-Shora and Metwally (2009). Protein content was determined according to Lowry *et al*. (1951).

Cotyledons of 5-day old seedlings of sunlower were subjected to treatment with 20-100 µM of $CdCl_2$ and $PbCl_2$ for 72 hours. Also, Cotyledons of 5-day old seedlings were subjected to treatment with NaCl (50-300 mM) for 72 hours. Hydrogen peroxide content and lipid peroxidation were determined according to Rao *et al.* (1997). All values are means of three measurements ± SE.

RESULTS AND DISCUSSION

The present results indicate that the tested growth regulators IAA, NAA and IPA induced acid phosphatase activity in *Helianthus annus* cotyledons (Fig 1).

In support indole-3-acetic acid (IAA) which stimulated ethylene production by enhancing 1-aminocyclopropane-1-carboxylate synthase activity. Also, it caused an increase in 1-aminocyclopropane-1-carboxylate oxidase transcript and activity levels (Peck and Kendle, 1994).

GA_3 other regulator stimulated the development of proteases in detached cotyledons **(**Ramakrishna and Rao, 2005**)**. Also, Taneyma *et al.* (1996) showed that endopeptidase activity was doubled when GA_3 was applied to the detached cotyledons of *Vigna mungo* seeds. The hormonal effect on acid phosphatase may be due to the induction of enzyme synthesis or suppression of the degradation of enzyme or both.

The effects of metal cations on the activity of acid phosphatase have been examined (Fig. 2). Enzyme was inhibited by Cd^{2+} and Pb^{2+} in sunflower cotyledons. Toxicity of heavy metals primarily results from their binding to sulphydryl groups in proteins or disruption of protein structure or displacement of an essential element (De Filippis and Pallaghy, 1994).

Cd^{2+} and Pb^{2+} enhanced lipid peroxidation (Figs. 3, 4) measured as total MDA content. MDA is the decomposition product of polyunsaturated fatty acids (PUFA) of biomembranes and its increase shows plants are under high-level antioxidant stress **(**Fadzilla *et al.,* 1997**)**. Cell membranes stability has been widely used to differentiate stress tolerant and susceptible cultivars of many crops and in some cases higher membrane stability could be correlated with better performance.

Although Cd^{2+} does not generate ROS directly, like other heavy metals such as Cu^{2+} and Fe^{3+}, it generates oxidative stress *via* interference with the antioxidant defense system **(**Sanita di Toopi and Gabrielli, 1999).

The accumulation of Cd^{2+} in plants could inhibit the uptake of Mg^{2+} and K^{+} on which protein synthesis system relied. However, the mechanism of Cd^{2+} inhibition of protein content is complex and need further study.

Under Cd^{2+} treatment, an increase in MDA content indicated the oxidative stress in cotyledons. MDA content, however, was lower in cotyledons pretreated with GA_3 (Fig. 5). Endogenous GA_3 plays an important role in protecting plants from oxidative stress.

Salinity treatment for the cotyledons resulted in the reduction of acid phosphatase activity (Fig. 6). These results are in agreement with those of Mohamed (2005). Pretreatment of cotyledons with 200 µM GA_3 resulted in partial alleviation of Cd^{2+} and Pb^{2+} toxicity. In support, GA_3 treatments overcame to variable extents the adverse effects of salinity stress (Tuna *et al.,* 2008).

In the present investigation salinity induced lipid peroxidation and increased H_2O_2 content (Fig. 7). It has been reported that membranes are the primary sites of salinity injury to cells and organelles because ROS can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids in plasmalemma or intracellular organelles (Candan and Tarhan, 2003).

Peroxidation of plasmalemma leads to the leakage of cellular contents, rapid desiccation and cell death. Intracellular membrane damage can affect respiratory activity in mitochondria, causing pigment to break down and leading to the loss of the carbon fixing ability in chloroplasts (Scandalios, 1993).

The disturbance of plant metabolism due to salinity is associated with damage caused by the superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) (Jebara *et al.,* 2005).

Free radicals disrupt normal metabolism through peroxidating lipids, which causes degradation and impairment of structural components leading to membrane leakage and changes in activities of enzymes bound to membranes (Bor *et al.,* 2003).

Cell membrane stability has been widely used to characterize stress tolerance, and high membrane stability is correlated with abiotic stress tolerance (Meloni *et al.,* 2003). Increased production of O_2^- and H_2O_2 in plants under salinity stress was associated with malondialdehyde (MDA) accumulation (Verma and Mishra, 2005).

COR alleviated partially the toxic effect of salinity (Fig. 8). COR has structural and functional similarity to jasmonic acid (Uppalapati *et al.,* 2005). COR is more biologically active than JAs (Tamogami and Kodama, 2000), so our results are a valuable support for the potential use of COR for plant growth regulation. COR could alleviate salinity stress through elevating the activity of antioxidative enzymes and limiting the generation of ROS.

Photosynthetic measurements indicate that an important role of JA in the adaptation of barley to a saline environment was through partial mitigation of the negative effects of salinity (Walia *et al.,* 2007).

Therefore, the present research detected very similar results of improved salt tolerance in sunflower cotyledons by COR treatment.

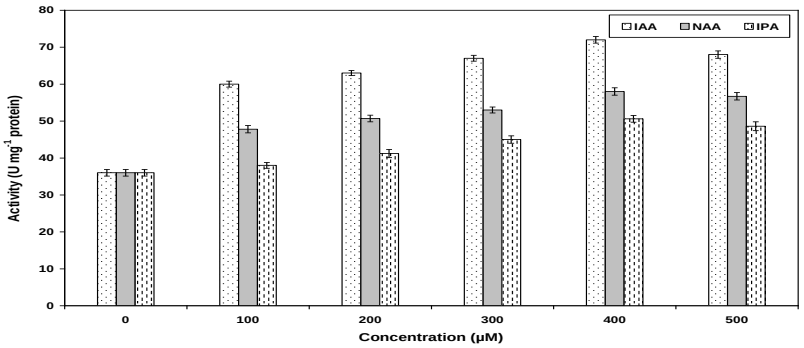

Fig 1: Effect of IAA, NAA and IPA on acid phosphatase activity.

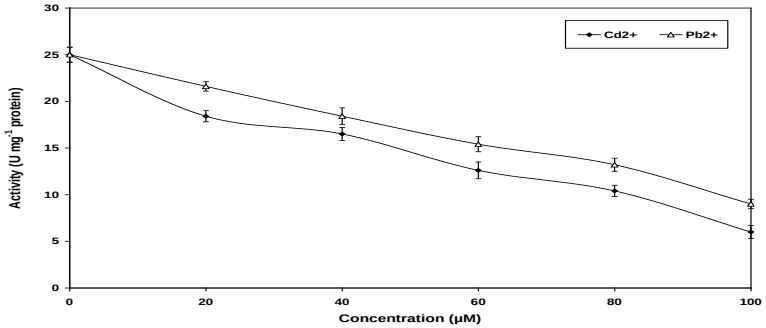

Fig 2: Effect of Cd^{2+} and Pb^{2+} on acid phosphatase activity.

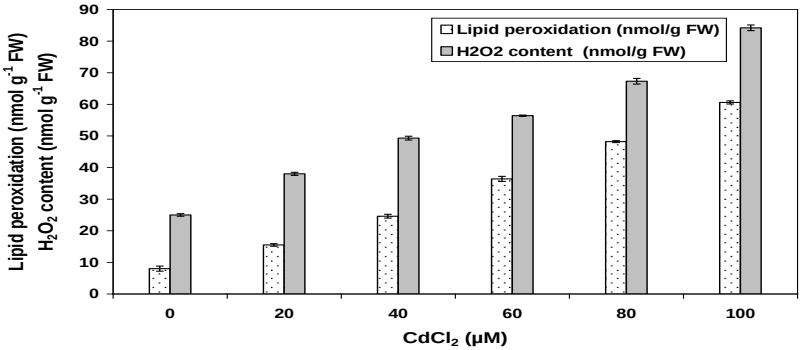

Fig 3: Effect of $CdCl_2$ on lipid peroxidation and H_2O_2 content.

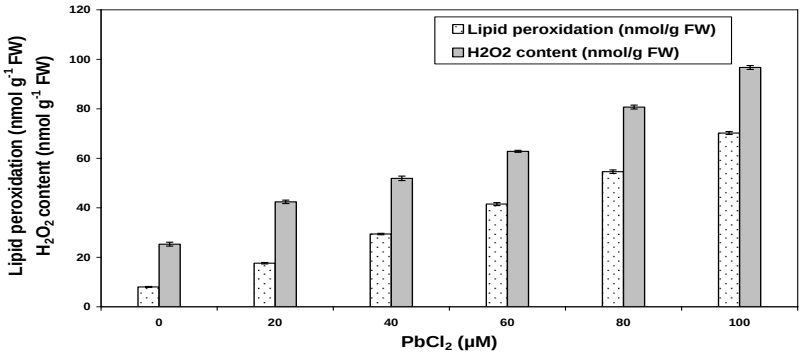

Fig 4: Effect of $PbCl_2$ on lipid peroxidation and H_2O_2 content.

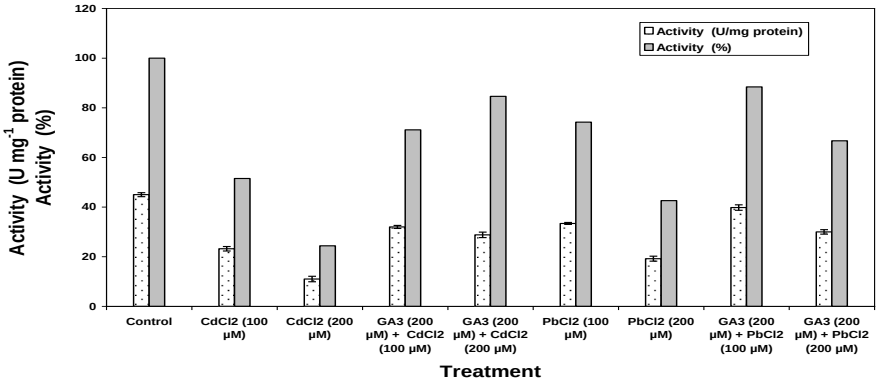

Fig 5: Alleviation of heavy metals ($CdCl_2$ and $PbCl_2$) toxicity on acid phosphatase activity by GA_3.

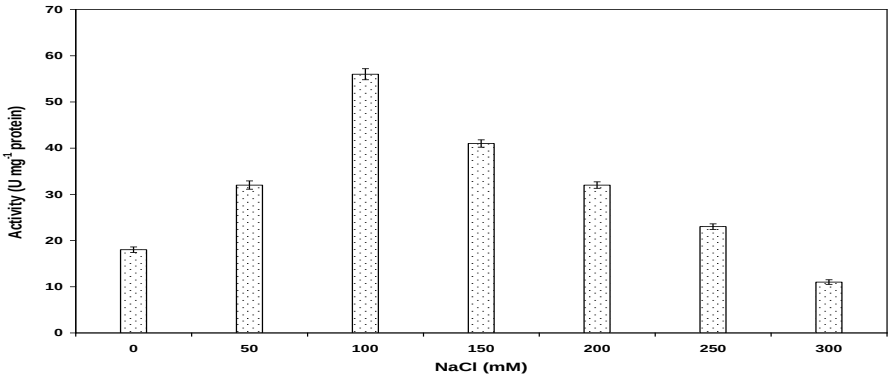

Fig 6: Effect of salinity stress on acid phosphatase activity.

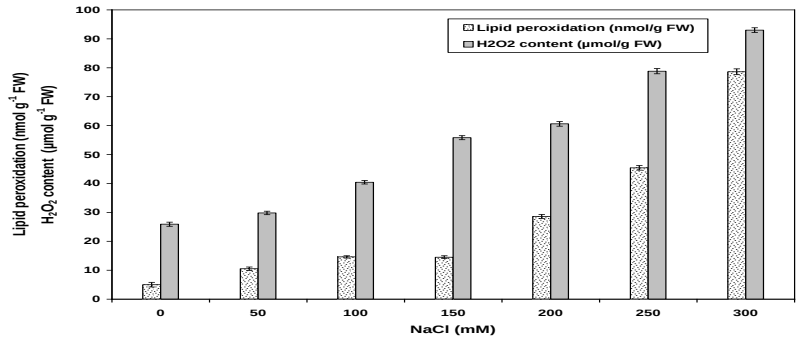

Fig 7: Influence of salt stress on lipid peroxidation and H_2O_2 content.

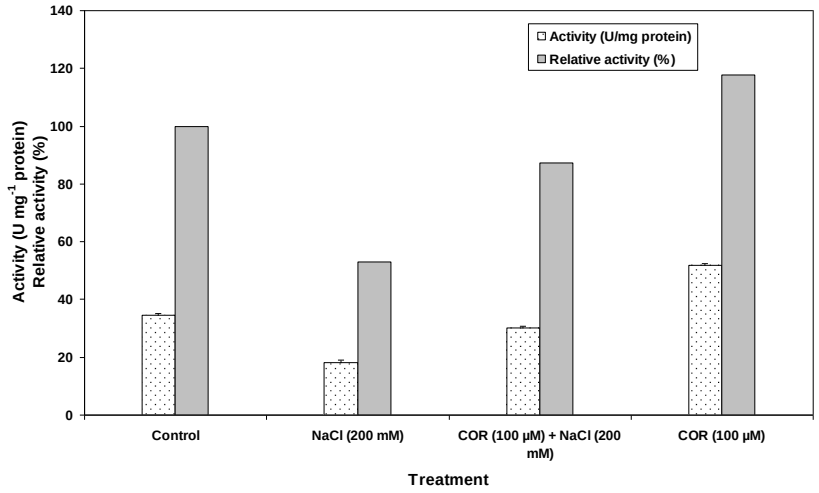

Fig 8: Effect of COR at 100 µM on acid phosphatase in cotyledons exposed to salinity stress (200 mM NaCl).

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تـأثير هرمونـات النمـو والملوحـه والعناصـر الثقيلـه علـى نشـاط إنـزيم الفوسـفاتيز الحامضى فى فلقات نبات دوار الشمس.

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هدف هذا البحث إلى دراسة تأثير بعض هرمونات النمو مثل اندول حمض الخليك و نافثيل حمض الخليك و اندول فينوكس حمض الخليك على نشاط إنزيم الفوسفاتيز الحامضى فى فلقات نبات دوار الشمس، وأظهرت النتائج أن معاملة الفلقات بكل من هذه المركبات على حده أدى إلى زيادة النشاط الإنزيمى زياده ملحوظه. أدت معاملة الفلقات بكل من كلوريد الكادميوم وكلوريد الرصاص إلى إنخفاض النشاط الإنزيمى وكذلك زيادة أكسدة الدهون وزيادة محتوى فوق أكسيد الهيدروجين. كما أظهرت معاملة الفلقات بحمض الجبريلليك قبل المعامله بكلوريد الكادميوم وكلوريد الرصاص إلى إنخفاض التأثير الضار لكل من المركبين. كما هدف هذا البحث إلى دراسة تأثير كلوريد الصوديوم عند تركيزات مختلفه (٥٠-٣٠٠ مللى مول) وأظهرت النتائج زيادة النشاط الإنزيمى عند التركيزات المنخفضه (٥٠-١٠٠ مللى مول) وانخفاض النشاط عند التركيزات المرتفعه (١٥٠-٣٠٠ مللى مول). كما أدت المعامله بكلوريد الصوديوم إلى زيادة أكسدة الدهون وزيادة محتوى فوق اكسيد الهيدروجين. وأظهرت النتائج أنه عند معاملة الفلقات بالكورونتين قبل المعامله بكلوريد الصوديوم قد أدى إلى إنخفاض أكسدة الدهون وإنخفاض محتوى فوق أكسيد الهيدروجين.