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EFFECT OF SALICYLIC ACID ON PIGMENT, PROTEIN CONTENT AND PEROXIDASE ACTIVITY IN EXCISED SUNFLOWER COTYLEDONS

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Abstract

Enviromental stress gives rise to the activation of adaptation and defence responses in plants. It is known that the role of salicylic acid (SA) is quite important in this mechanism. In this study its effect was investigated on excised cotyledons of sunflower (*Helianthus annuus* L.) seedlings. The sunflower seedlings were grown in dark conditions for 9 days and then their etiolated cotyledons were harvested. Then, they were transferred into Petri dishes containing 0.001 μM, 0.1 μM, 10 μM, 1000 μM SA. Cotyledons were incubated for 14 hours in the dark at room temperature, then they were incubated in light period for 3 hours. Chlorophyll, carotenoid content, protein amount and peroxidase (POD) activity in the cotyledons was examined. SA 1000 μM solution showed the toxic effect in growth considering the results of total chlorophyll, carotenoid content and protein amount. An increasing 2 fold of chlorophyll content in 10 μM SA and 3.5 fold of carotenoid content in 0.1 μM SA treated cotyledons comparing to the control were observed. Protein amount increased in all concentrations except 1000 μM SA. POD activity was also stimulated in all concentration of SA solutions. However, the clear difference in 0.001 μM SA was not seen. As a result, chlorophyll, carotenoid, protein contents and POD activity increased in exogenic SA applications

Introduction

Salicylic acid (SA) is a potent signaling molecule in plants and is involved in eliciting specific responses to biotic and abiotic stresses (Krantev *et al*., 2006). SA has been identified as a signalling component in numerous plant responses to stres, including UV-B (Surplus *et al*., 1998), exposure to ozone (Rao & Davis, 1999) and pathogen attack (Gaffney *et al*., 1993; Glazebrook, 1999). SA is also involved in activation of the stress-induced antioxidant system when plants are exposed to stres, and is now considered to be a hormonal substance that plays a key role in regulating plant growth and development (Huang *et al*., *2008*). Since the discovery that salicylic acid (SA) is produced upon infection of cucumber (Métraux *et al*., 1990) or tobacco (Malamy *et al*., 1990) leaves prior to the expression of systemic resistance, much efort has been devoted to demonstrate the role of SA in the resistance of plants to diseases (Raskin, 1992; Delaney *et al*., 1994). Originally SA was extracted from the willow bark to make the well known pain relief medication Aspirin. SA is thought to promote disease resistance, increase flower life, inhibit seed germination and SA is accepted as a natural plant growth regulator (Raskin, 1992; Chen&Kuc, 1999).

A role for SA in plant growth and development, flowering, ion uptake, stomatal regulation and photosynthesis has been investigated (Pancheva *et al*., 1996; Popova *et al*., 1997; Uzunova&Popova 2000). Intracellular SA concentration and SA signalling pathway(s) are associated with the functions controlling cell growth, cell death and defence (Chen *et al*., 2001; Tronchet, 2001). Stem diameter and height of the plants are increased by 10^{-10} and 10^{-8} M SA. Similarly, applications of 10^{-8} and 10^{-6} M SA increased fresh stem weight, dry stem weight and root length (San-Miguel *et al*., 2003).

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The exogenous application of SA to plants results in a range of physiological responses: i.e., inhibition of ethylene biosynthesis and seed germination (Leslie&Romani, 1988); interference with the ion transportation and absorption in the membranes of root cells (Harper&Balke, 1981); reversal of abscisic acid effects in leaf abscission and inhibition of plant growth (Raskin 1995).

In the present study, the effect of SA on excised cotyledon growth in four SA concentration (0.001 μM, 0.1 μM, 10 μM, 1000μM) for comparison was examined.

Materials and Methods

Experimental Design: Sunflower (*Helianthus annuus* L.) seeds were planted in sawdust and they were grown at 25±2^{o}C in darkness to prevent any chlorophyll formation in the cotyledons. Under a dim green light two cotyledons were excised from several uniform seedlings. Three cotyledons pairs were placed in 5 cm diameter Petri dishes already containing 5 ml of experimental solution. The cotyledons were allowed to imbibe the SA solution in various concentrations in darkness for 14 hours at room temperature and they were placed in to the light for 3 h.

Chlorophyll determination: Pigment was extracted by grinding the cotyledons of sunflower in 90% acetone (v/v) and the total chlorophyll and carotenoid content determined spectrophotometrically (Shimadzu 1601),(Parsons&Strickland,1963).

Extraction of protein: The cotyledon samples were homogenized with ice-cold 0.1 mM Sodium phosphate buffer (pH 6.8). The homogenates were centrifuged at 13,000 rpm for 30 min at 4 ^{o}C and supernatants were used for determination of total soluble protein content and total peroxidase enzyme assays. Protein content of the extracts were determined according to Bradford (1976) using bovin serum albumin as standard.

Peroxidase activity assay: The reaction mixture consisted of 0.25% (v/v) guaiacol in 1 ml 0.1 M Sodium phosphate buffer, pH 7.0, containing 0.1% hydrogen peroxide. Crude enzyme extract 60 μl was added to initiate the reaction which was measured spectrophotometrically at 470 nm due to the guaiacol oxidation which was recorded for 2 min., and defined quantitatively as ΔA/g.Fr.W.xMin (Birecka *et al*., 1973).

Statistical analysis: Each treatment was analysed with at least 3 replicate tissue samples bulked at least 20 plants. The data presented here are the mean values ± SE of three independent experiments. Comparison with $p<0.05$ were considered significantly different.

Results

Fig. 1 and Fig. 2 summarize the chlorophyll and carotenoid content of the cotyledons excised from 9-day old seedlings and incubated under light for 3 h in both control and different SA (0.001, 0.1, 10, 1000 μM) concentrations. Chlorophyll content 1.5 fold increase in 10 μM SA and 3.4 fold increase of carotenoid content in 10^{-7} M SA treatments comparing with the control cotyledons were observed. Fig. 3 shows histograms of the total protein amounts. According to the obtained findings, protein amount was increased @ 1.9, 2.3 and 1.7 fold in 0.001, 0.1 and 10 μM, respectively. The lowest total protein content was found at 1000 μM, and the highest at 0.1 μM respectively, when compared with the control group. Fig. 4 shows histograms from the data of POD activity belonging to the control and experimental groups. The POD activity of the control significantly

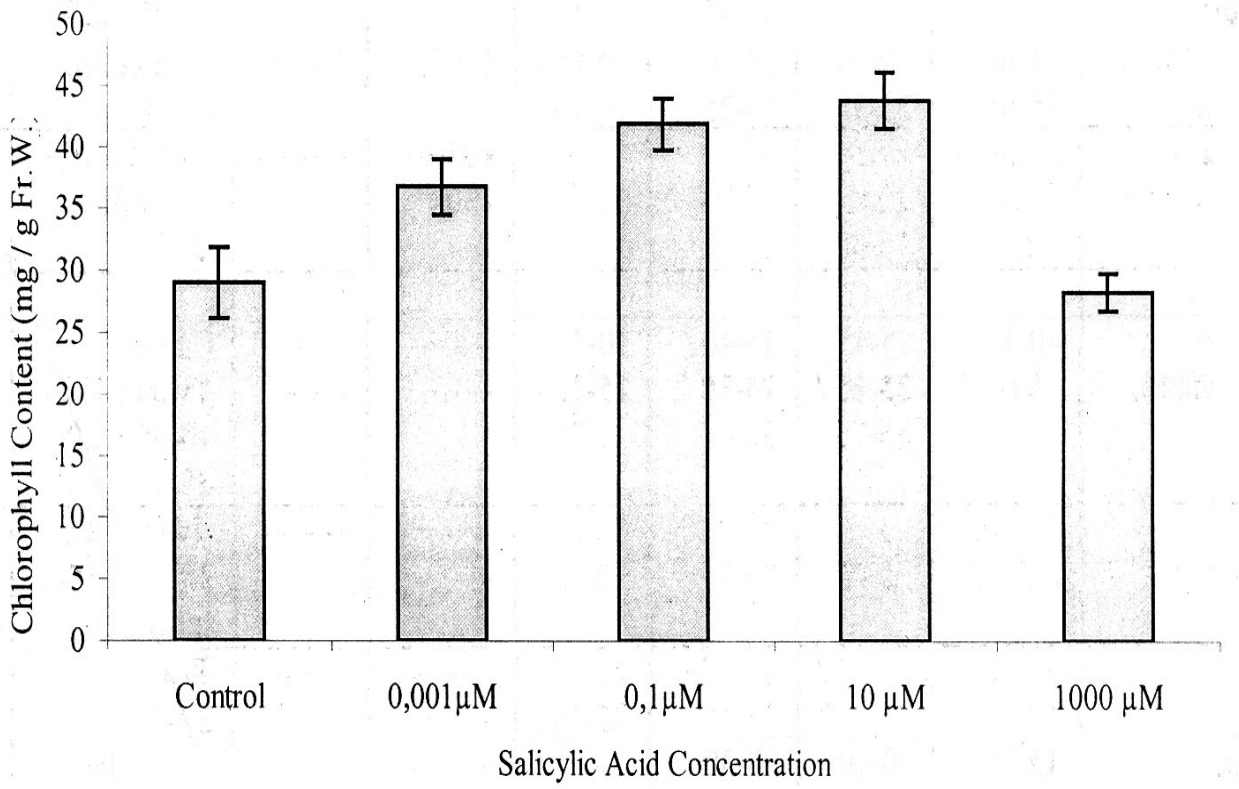

Fig. 1. Effect of different concentrations of salicylic acid on chlorophyll contents in sunflower cotyledons.

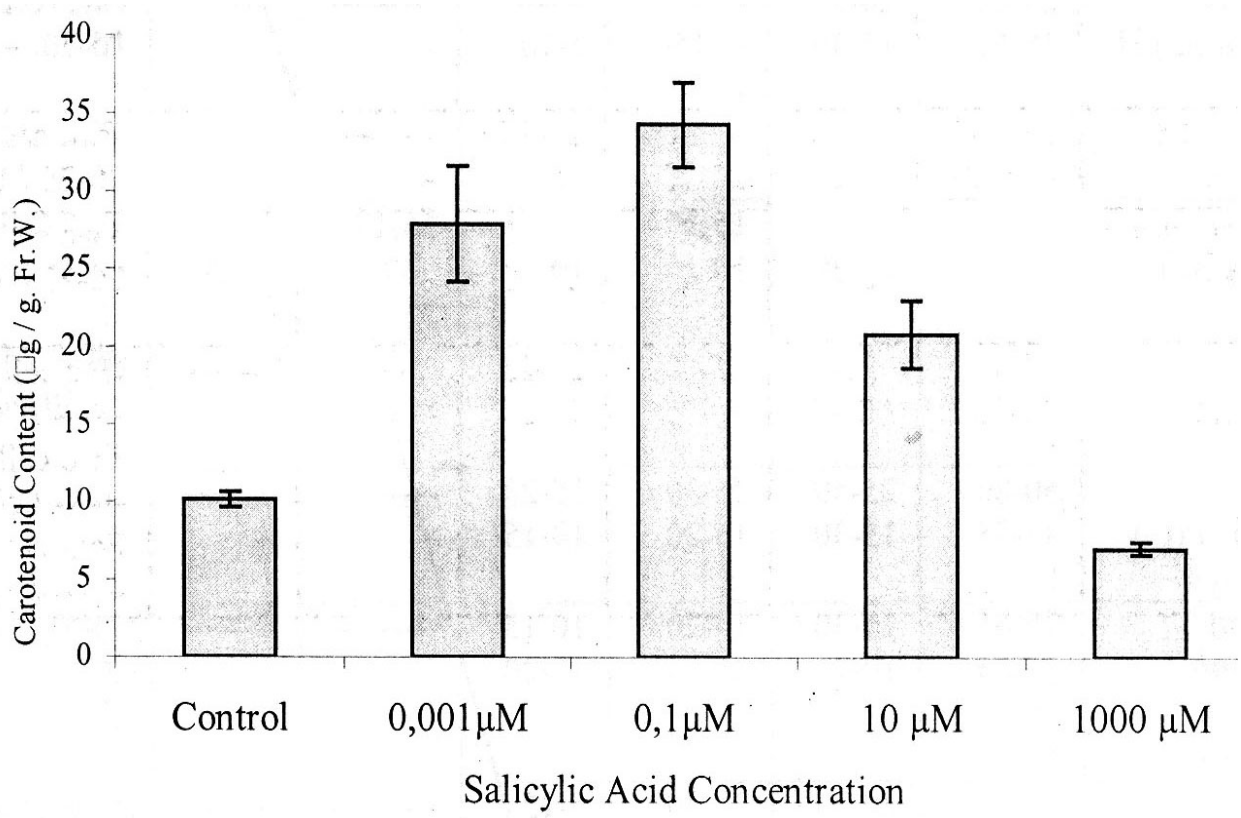

Fig. 2. Effect of different concentrations of salicylic acid on carotenoid contents in sunflower cotyledons.

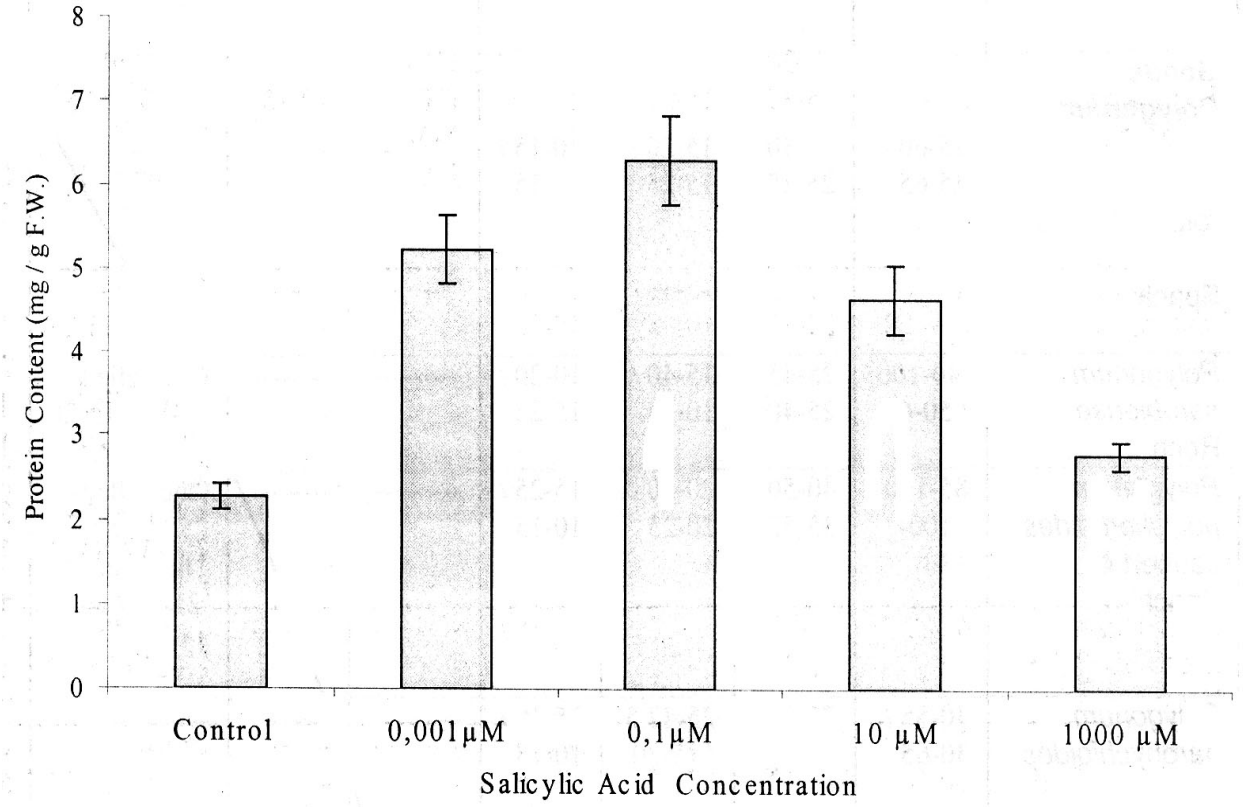

Fig. 3. Effect of different concentrations of salicylic acid on soluble protein contents in sunflower cotyledons.

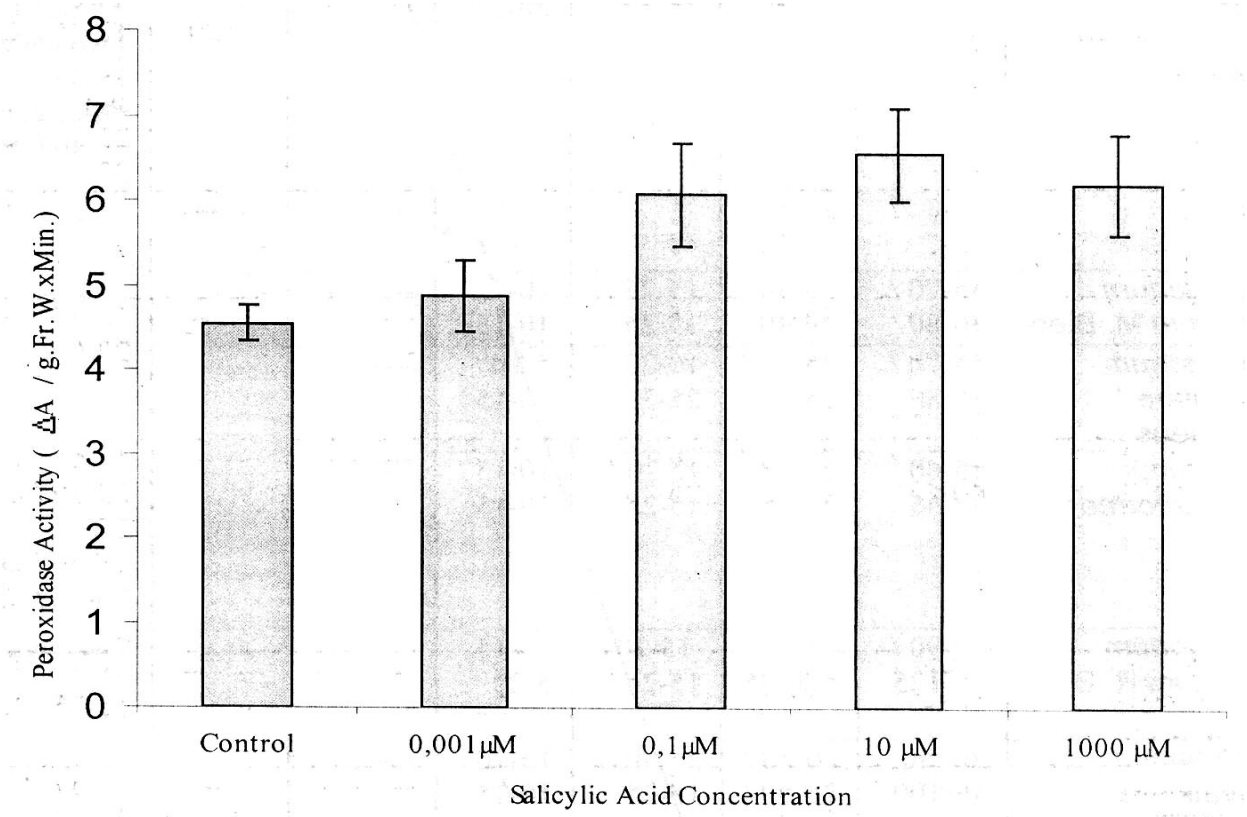

Fig. 4. Effect of different concentrations of salicylic acid on peroxidase activity in sunflower cotyledons.

increased as a reaction to the stress of injury due to excision. The POD activity of sunflower cotyledons was stimulated in all concentration of SA solutions however the clear difference between these concentrations was not seen.

As a result, chlorophyll, carotenoid, protein contents and POD activity were increased in exogenic SA applications.

Discussion

The responses of growth are most important phenomenons of plant physiology. In growth, which is a result of accelerated anabolic reactions in the cells and consequent lipid, protein, chlorophyll, DNA and RNA synthesis were studied in detail. While low concentrations of cytokinin, gibberellin, auxin and brassinosteroid accelerate growth (Hare, 1984; Jang *et al*., 2000) ethylene, abscisic acid and jasmonic acid retard it (Kang *et al*., 2005; Wilson, 2007). The endogenous polyamin (PA) levels were higher in the treatments with SA and lower in the treatments with MeJA.

It has been known that increase of chlorophyll occurs during growth. In this study, chlorophyll content also increased sunflower cotyledons incubated in SA concentrations. In the germinated seeds, enzyme activities increase from very low amounts to peak levels during growth (Matsui *et al*., 1999). Altman (1982) showed that ethylene accelerated chloropyll loss. Li *et al*., (1992) established that SA inhibited the activity of ACC synthase enzyme, preventing the formation of ethylene and chlorophyll loss. In this research, we found increase in the chlorophyll content compared to the control assosiated with treatment of decreasing SA concentrations. While 10 and 0.1 μM SA concentrations accelerated carotenoid content of excised cotyledons, 1000 μM SA retarded it.

Researhers investigating physiological changes that occur in cotyledons of various plants during growth observed that proteins were synthesized (Palavan-Ünsal *et al*., 2002). In this study, a gradual increase in the total protein content by 1.9, 2.3 and 1.7 fold in 0.001, 0.1 and 10 μM respectively, compared to the control was observed.

Sakhabutdinova *et al*., (2003) estabilished that treatment of wheat plants with 0.05 M SA increased the level of cell division within the apical meristem of seedlings roots which caused an increase in plant growth. We have determined that POD activity gradually decreases by the application of decreasing SA concentration in sunflower cotyledons. It would suggest that POD activity decreases during growth period. It would suggest that growth promoting effect of SA are due to the phenomenon described above. The results of this research is also exhibited SA as plant growth regulator.

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