

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

SERAP ÇAG, GÜL CEVAHIR-ÖZ, MINE SARSAG AND NIHAL GÖREN-SAGLAM

*Department of Biology,
Botany Section, Istanbul University, Istanbul-Turkey*

Abstract

Environmental 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 μ M, 0.1 μ M, 10 μ M, 1000 μ M 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 μ M 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 μ M SA and 3.5 fold of carotenoid content in 0.1 μ M SA treated cotyledons comparing to the control were observed. Protein amount increased in all concentrations except 1000 μ M SA. POD activity was also stimulated in all concentration of SA solutions. However, the clear difference in 0.001 μ M 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 stress, 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 stress, 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 effort 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).

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 μ M, 0.1 μ M, 10 μ M, 1000 μ M) for comparison was examined.

Materials and Methods

Experimental Design: Sunflower (*Helianthus annuus* L.) seeds were planted in sawdust and they were grown at $25\pm 2^\circ\text{C}$ 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°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 μ l 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 $\Delta A/g.Fr.W.xMin$ (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 \pm 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 μ M) concentrations. Chlorophyll content 1.5 fold increase in 10 μ M 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 μ M, respectively. The lowest total protein content was found at 1000 μ M, and the highest at 0.1 μ M 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

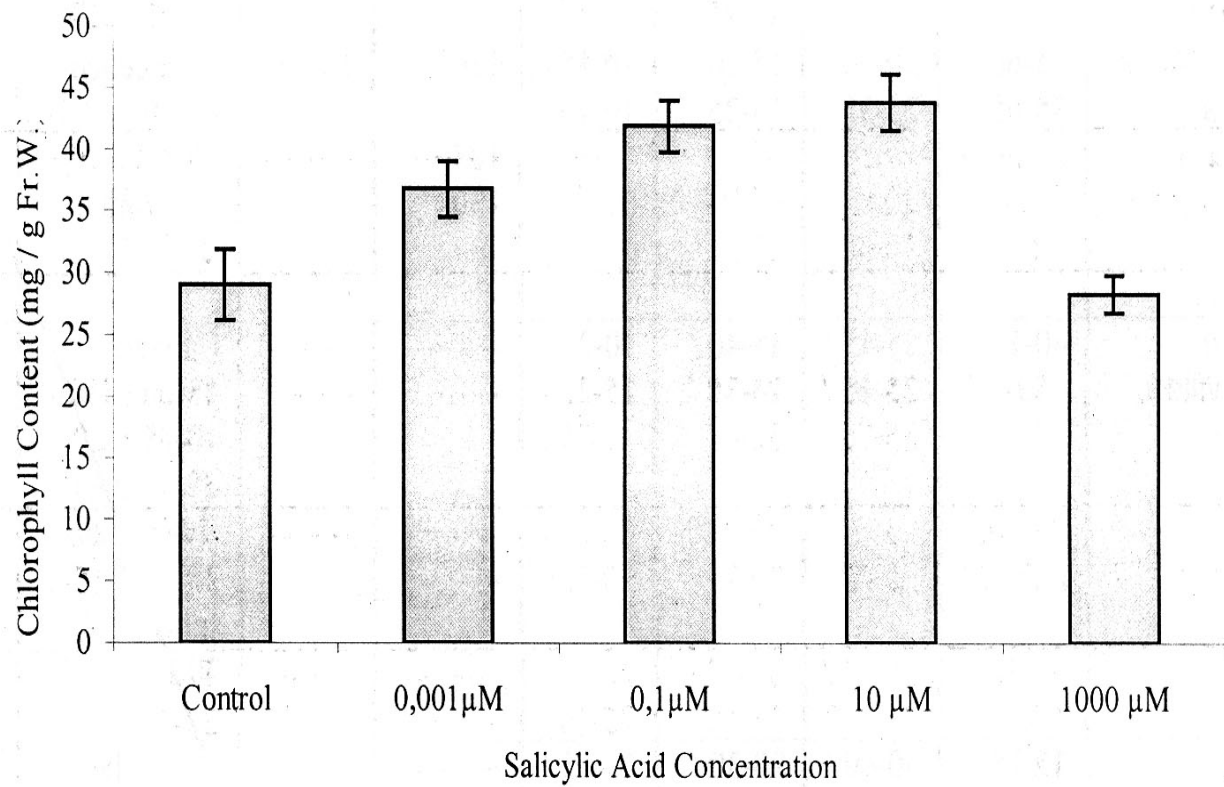


Fig. 1. Effect of different concentrations of salicylic acid on chlorophyll contents in sunflower cotyledons.

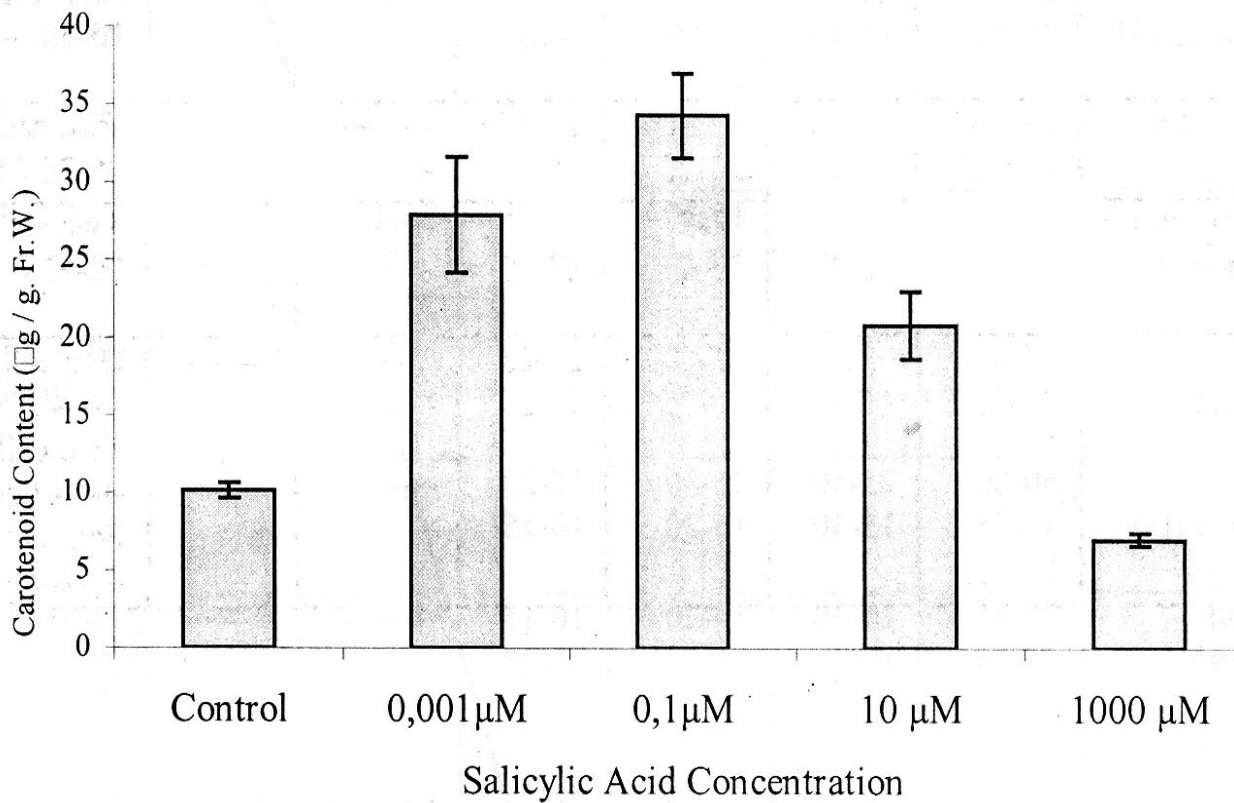


Fig. 2. Effect of different concentrations of salicylic acid on carotenoid contents in sunflower cotyledons.

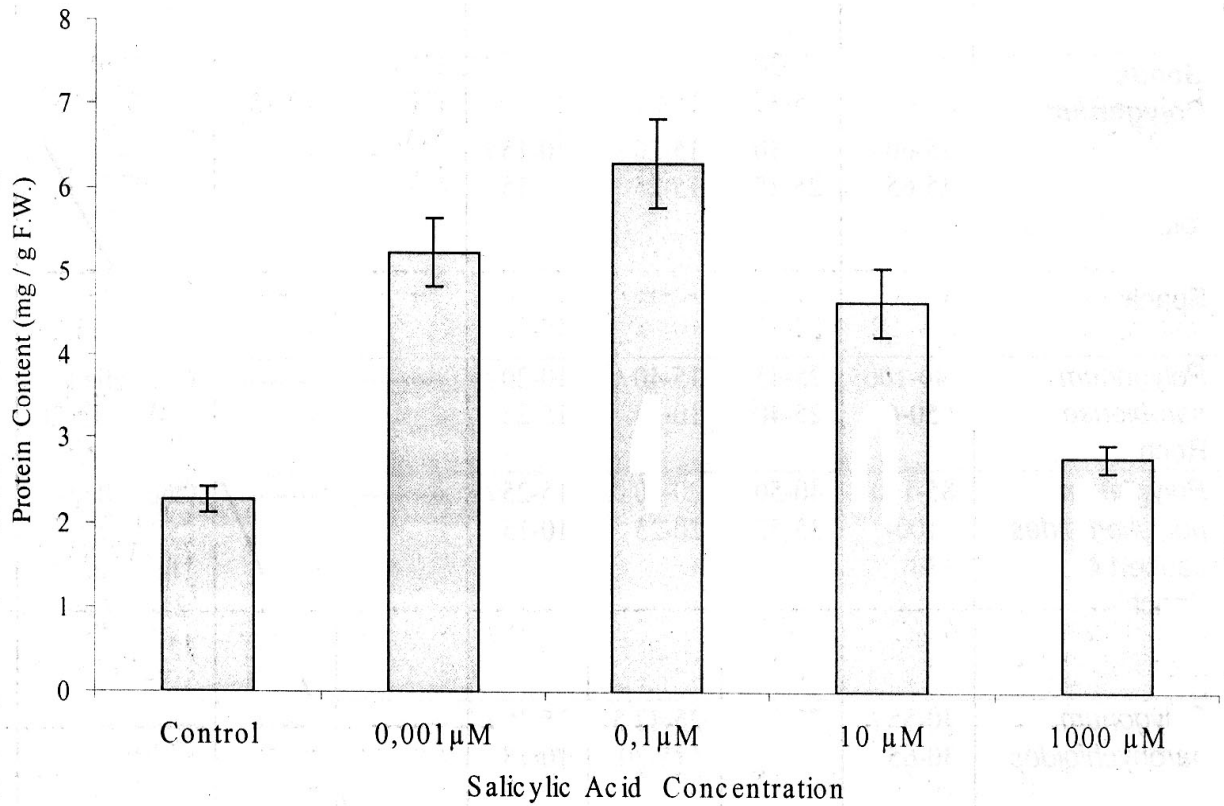


Fig. 3. Effect of different concentrations of salicylic acid on soluble protein contents in sunflower cotyledons.

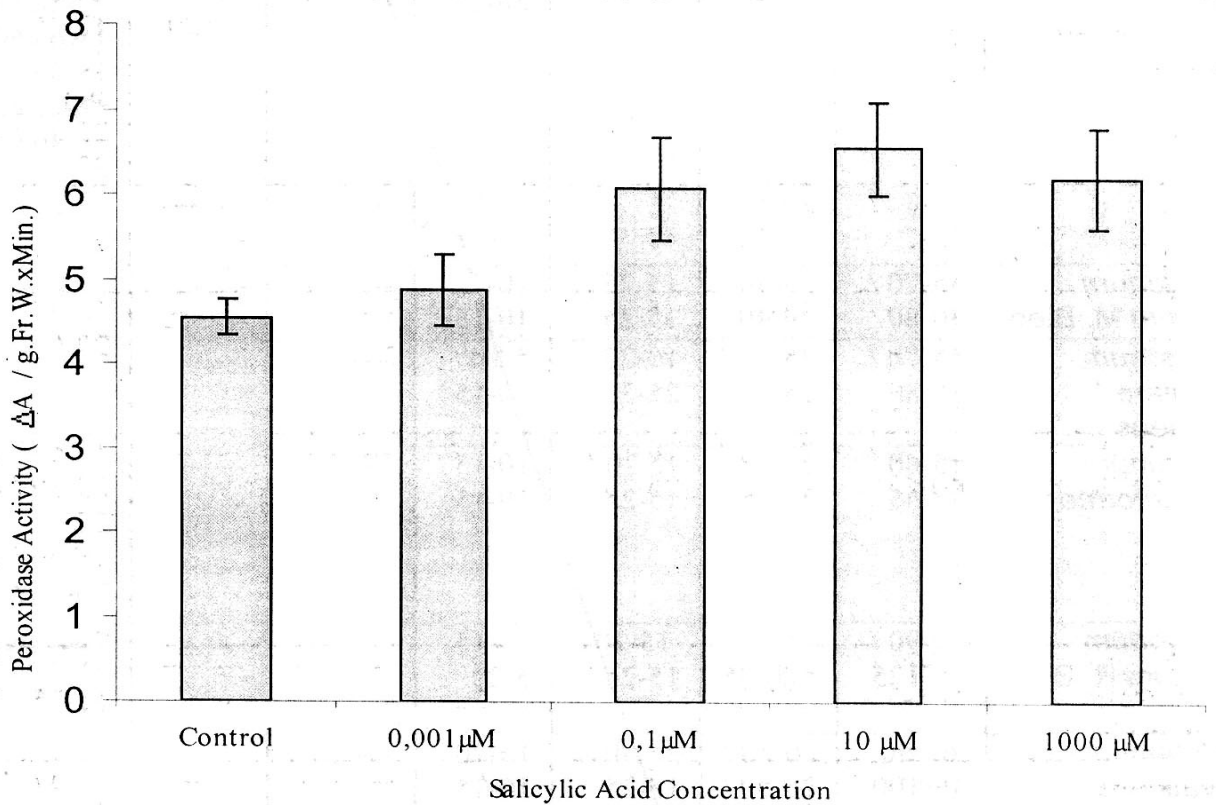


Fig. 4. Effect of different concentrations of salicylic acid on peroxidase activity in sunflower cotyledons.

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 chlorophyll 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 associated 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.

Researchers 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) established 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.

References

- Altman, A. 1982. Retardation of radish leaf senescence by polyamines. *Plant Physiology*, 54:189-193.
- Chen, H., C. Hou, J. Kuc and Y. Lin. 2001. Ca^{2+} -dependent and Ca^{2+} -independent excretion modes of salicylic acid in tobacco cell suspension culture. *Journal of Experimental Botany*, 52: 1219-1226
- Chen, H. and J. Kuc. 1999. Ca^{2+} -dependent excretion of salicylic acid in tobacco cell suspension culture. *Botanical Bulletin Acad. Sin.*, 40: 267-273
- Delaney, T.P., S. Uknes, B. Vernooij, L. Friedrich, K. Weymann, D. Negrotto, T. Gaffney, M. Gutrella and H. Kessmann. 1994. A central role of salicylic acid in plant disease resistance. *Science*, 266: 1247-1250
- Gaffney, T., L. Friedrich, B. Vernooij, D. Negrotto, G. Nye, S. Uknes, E. Word, H. Kessman and J. Ryals. 1993. Requirement of salicylic acid for the induction of systemic acquired-resistance. *Science*, 261: 754-756.

- Galzebrook, J. 1999. Genes controlling expression of defence responses in *Arabidopsis*. *Current Opinion in Plant Biology*, 2: 280-286.
- Hare, C.R. 1984. Stimulation of early height growth in longleaf pine with growth regulators. *Canadian Journal of Forest Research*, 14: 459-462.
- Harper, J.R. and N.E. Balke. 1981. Characterization of the inhibition of K⁺ absorption in oat roots by salicylic acid. *Plant Physiology*, 68: 1349-1353.
- Huang, R.-H., J.-H. Liu, Y.-M. Lu and R.-X. Xia. 2008. Effect of salicylic acid on the antioxidant system in the pulp of 'Cara cara' navel orange (*Citrus sinensis* (L.) Osbeck) at different storage temperatures. *Postharvest Biology and Technology*, 47: 168-175.
- Jang, J.-C., S. Fujioka, M. Tasaka, H. Seto, A. Ishii, M. Aida, S. Yoshida and J. Sheen. 2000. A critical role of sterols in embryonic patterning and meristem programming revealed by the fackel mutants of *Arabidopsis thaliana*. *Genes and Development*, 14: 1485-1487.
- Kang, D.-J., Y.-J. Seo, J.-D. Lee, R. Ishii, K.U. Kim, D.H. Shin, S.K. Park, S.W. Jang and I.J. Lee. 2005. Jasmonic acid differentially affects growth, ion uptake and Abscisic Acid concentration in salt-tolerant and salt-sensitive rice cultivars. *Journal of Agronomy and Crop Science*, 191: 273-282.
- Krantev, A., R. Yordanova and L. Popova. 2006. Salicylic acid decreases Cd toxicity maize plants. *General and Applied Plant Physiology*, Special Issue, 45-52.
- Leslie, C.A. and R.J. Romaani. 1988. Inhibition of the ethylene biosynthesis by salicylic acid. *Plant Physiology*, 88: 833-837.
- Li, N., B.L. Parsons, D.R. Liu and A.K. Mattoo. 1992. Accumulation of wound-inducible ACC synthase transcript in tomato fruit is inhibited by salicylic acid and polyamines. *Plant Molecular Biology*, 18: 477-487.
- Malamy, J., J.P. Carr, D.F. Klessig and I. Raskin. 1990. Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection. *Science*, 250:1002-1004.
- Matsui, K., K. Hijiyama, Y. Tabuchi and T. Kajiwara. 1999. Cucumber cotyledon lipoxygenase during postgerminative growth. Its expression and action on lipid bodies. *Plant Physiology*, 119: 1279-1288.
- Métraux, J.P., H. Signer, J. Ryals, E. Ward, M. Wyss-Benz, J. Gaudin, K. Raschdorf, E. Schmid, W. Blum and F. Inverardi. 1990. Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science*, 250:1004-1006.
- Palavan-Ünsal, N., S. Çag and E. Çetin. 2002. Growth responses of excised radish cotyledons to meta-topolin. *Canadian Journal of Plant Science*, 82: 191-194.
- Pancheva, T.V., L.P. Popova and A.N. Uzunova. 1996. Effects of salicylic acid on growth and photosynthesis in barley plants. *J. Plant Physiol.*, 149: 57-63.
- Parsons, T.R. and J.D.H. Strickland. 1963. Discussion of spectrophotometric determination of marine pigments, with revised equations for ascertaining chlorophylls and carotenoids. *Journal of Marine Research*, 21: 115-163.
- Popova, L.P., T.V. Pancheva and A.N. Uzunova. 1997. Salicylic acid: Properties, biosynthesis and physiological role. *Bulgarian Journal of Plant Physiology*, 23: 85-93.
- Rao, M.V. and K.R. Davis. 1999. Ozone-induced cell death via two distinct mechanisms in *Arabidopsis*: the role of salicylic acid. *Plant Journal*, 17: 603-614.
- Raskin, I. 1992. The role of salicylic acid in plants. *Annual Review Plant Physiology and Plant Molecular Biology*, 43: 439-463.
- Raskin, I. 1995. Salicylic acid. In: *Plant hormones and their role in plant growth and development*, 2nd Edition. (Ed.): P.J. Davies. Kluwer Academic Publishers, Dordrecht. pp. 188-205.

- Sakhabutdinova, A.R., D.R. Fatkhutdinova, M.V. Bezrukova and F.M. Shakirova. 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulgarian Journal of Plant Physiology*, Special Issue, 314-319.
- San-Miguel, R., M.O. Gutiérrez and A. Larqué-Saavedra. 2003. Salicylic acid increases the biomass accumulation of *Pinus patula*. *Southern Journal of Applied Forestry*, 27(1): 52-54.
- Surplus, S.L., B.R. Jordan, A.M. Murphy, J.P. Carr, B.Thomas and S.A.H. Mackerness. 1998. Ultraviolet-B induced responses in *Arabidopsis thaliana*: role of salicylic acid and reactive oxygen species in the regulation of transcripts encoding photosynthetic and acidic pathogenesis related proteins. *Plant, Cell and Environment*, 21: 685-694.
- Tronchet, M., B. Ranty, Y. Marco and D. Roby. 2001. *HSR203* antisense suppression in tobacco accelerates development of hypersensitive cell death. *The Plant Journal*, 27: 115-127.
- Uzunova, A.N. and L.P. Popova. 2000. Effect of salicylic acid on leaf anatomy and chloroplast ultrastructure of barley plants. *Photosynthetica*, 38: 243-250.
- Wilson, C. 2007. effect of Jasmonic Acid on growth and ion relations of *Oryza sativa* L., grown under salinity stress. *American Society of Agronomy Meetings*. pp. 63-14.

(Received for publication 7 May 2008)