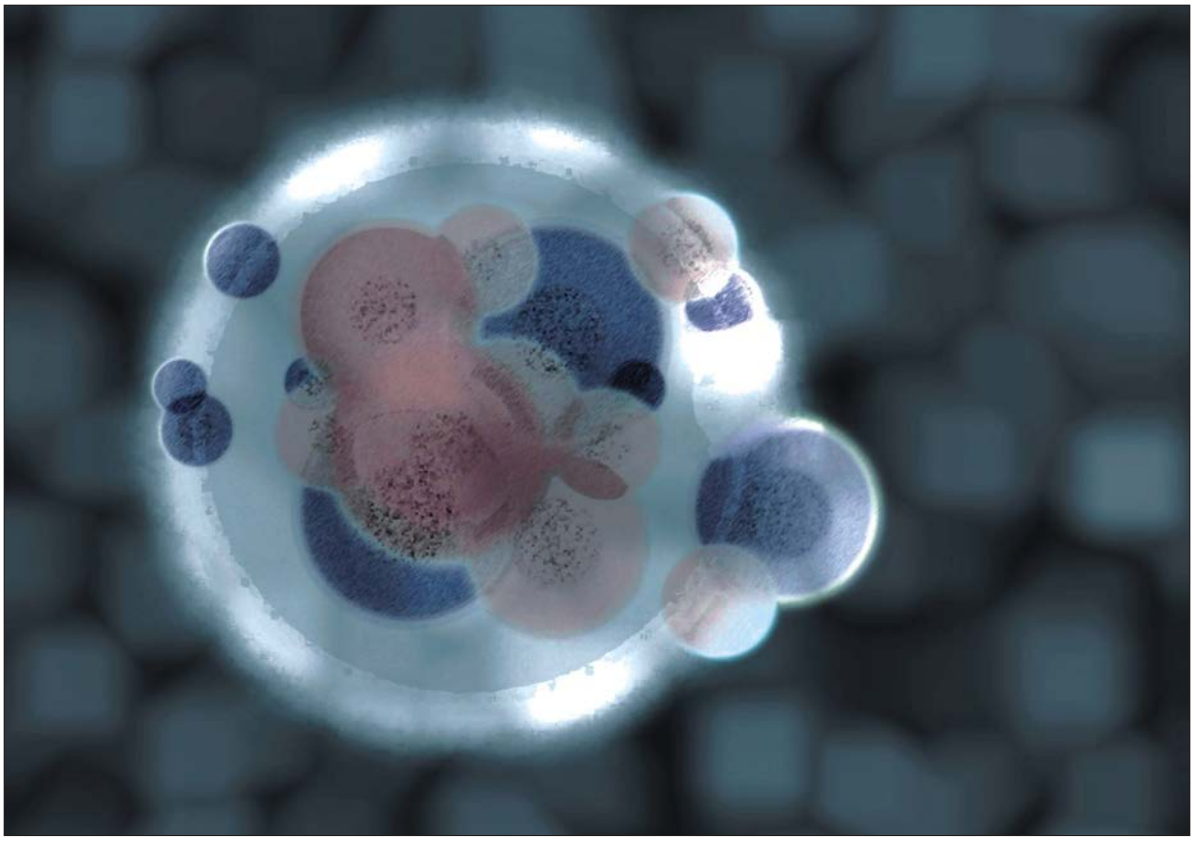

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Effects of Salicylic Acid on Fresh Weight Change, Chlorophyll and Protein Amounts of Radish (*Raphanus sativus* L.) Seedlings

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Abstract: This study examined the effects of various concentrations (0, 0.2, 1 and 2 mM) of salicylic acid on fresh weight change, pigment and protein amounts of ten-day-old radish (*Raphanus sativus* L. cv. White) seedlings grown under controlled conditions. The solutions were applied to the roots of one-week-old seedlings for 3 days through an enclosed system. According to the results, it was found that 0.2 mM SA did not affect fresh weight gain, pigment and protein amounts of the seedlings, that 1 and 2 mM SA administration prevented fresh weight gain and additionally, that these two concentrations significantly reduced chlorophyll (a+b) and protein amounts of the seedlings. It was concluded that high concentrations of salicylic acid produced osmotic and toxic stress in radish seedlings, thereby preventing fresh weight gain and reducing pigment and protein amounts.

Key words: Salicylic acid, radish, fresh weight, chlorophyll, protein

INTRODUCTION

The commercially available form of salicylic acid, which is a secondary plant product, is acetylsalicylic acid (ASA). It is known that in aqueous solutions, ASA is hydrolyzed almost entirely to SA, which is its active ingredient (Mitchell and Broadhead, 1967). Depending on its concentration, SA performs important actions in the growth and development processes of plants. These actions include exercising a thermogenic effect (Raskin, 1995), increasing thermotolerance (James *et al*., 1998), stimulating adventive root formation (Riov and Yang, 1989), having a herbicidic effect (Shettel and Balke, 1983), reducing leaf shed (Ferrarese *et al*., 1996), providing resistance against pathogens (Salisbury and Ross, 1992), inhibiting ethylene biosynthesis (Romani *et al*., 1989; Huang *et al*., 1993) and changing the quality and quantity of proteins (Jung *et al*., 1993). It has been claimed (Ray, 1986) that SA and similar phenolic compounds exercise their effect of providing resistance against different stress factors in plants (Bergmann *et al*., 1994; Ágnes *et al*., 2005) by modifying the effects of abscisic acid (Apte and Laloraya, 1982) and cytokinins (Ray *et al*., 1983). These examples, as well as many other physiological effects brought about by SA invoked in several researchers the idea that this substance might be a new plant growth regulator (Raskin, 1995; Losanka *et al*., 1997; Rajasekaran and Balke, 1998, 1999).

SA is known to increase nitrate reductase activity (NRA) in corn (*Zea mays* L. cv.) seedlings (Raskin, 1995). It was purported that SA could exercise such an effect on NRA in the mediation of plant hormones (Schneider and Whitman, 1974). It is known that administration of cytokinins to the leaves of some one-year-old plants delays loss of chlorophyll and improves protein synthesis (Osborne, 1965; Parthier, 1964). Besides, cytokinins are known to have a delaying effect on proteolytic activity and leaf senescence (Noodén *et al*., 1997). It is known at present that plants under stress age more rapidly than those under optimum conditions (Vaadia *et al*., 1961). It has been argued that the decline in plastid formation and chlorophyll-carotenoid synthesis in plants exposed to stress results from the accumulation of abscisic acid (Duysen and Freeman, 1976) or the decrease in cytokinins levels (Itai and Ben-Zioni, 1973). It was understood in a study conducted in corn protoplast culture that ASA was an effective inhibitor of ethylene biosynthesis (Carswel *et al*., 1989). In another study carried out on discs of plucked rice leaves, ethylene biosynthesis was found to be inhibited in 2 h following SA administration (Huang *et al*., 1993). In still another study performed in radish (*Raphanus sativus* L.) cotyledons, ethylene accumulation stimulated chlorophyll degradation (Knee, 1991).

There is not sufficient information on how SA influences weight change in plants and what changes it brings about in pigment and protein contents of plants in a concentration-dependent way. The fact that effects of ASA on plants are similar to those of SA requires us to take studies using ASA in consideration as well (Mitchell and Broadhead, 1967). SA was observed to reduce leaf area (secondary leaf), root growth, as well as protein and

chlorophyll (a+b) amount parallel to an increase in its concentration in barley plants, which were developed from barley seeds germinated in SA solutions of varying concentrations and grown in SA-containing milieu (Pancheva *et al*., 1996). In a study Wajahatullah *et al*. (2003) found that spraying minute concentrations (10^{-5} mol L^{-1}) of SA and ASA on the leaves led to an increase in the overall photosynthetic yield of soy bean and corn. It was reported in the same study that stomatal mobility and transpiration increased, while chlorophyll amount remained unchanged. In studies where one-week-old corn and bean seedlings were used ASA administration (50, 250 and 1000 ppm) to the root (Çanakçı and Munzuroğlu, 2002) or the leaf (Çanakçı and Munzuroğlu, 2000) caused an increase in fresh weight loss, dry weight gain and a decrease in transpiration in high concentrations. It was reported in a study using discs obtained from primary leaves of one-month-old bean seedlings that chlorophyll a and b amount decreased, carotenoid amount remained unaffected, while fresh weight loss and protein destruction increased parallel to the increase in ASA concentration (100, 250 and 500 ppm) (Çanakçı, 2003). ASA (Çanakçı and Munzuroğlu, 2002, 2000) and SA (Manthe *et al*., 1992) was found to lead to the closure of stomata pores in high concentrations.

Plants under stress experience early aging after significant metabolic changes like a decrease in protein synthesis or an increase in protein destruction and chlorosis.

MATERIALS AND METHODS

Preparation of plant materials and salicylic acid solutions: This study used one-week-old seedlings developed from the germination of radish (*Raphanus sativus* L. cv. White) seeds as the vegetable material. For this purpose, radish seeds were left to swell in tap water at 25±2°C for 4 h. Then, the seeds were germinated upon cotton wetted with tap water at 25±2°C in dark. Of the germinated seeds, homogeneous ones were chosen 44 h after they were wetted and transplanted between the folds of wet filter papers in 100 mL beakers containing 50 mL Hoagland nutrient solution. These were kept under controlled conditions until they were one-week old. Solutions of crystal SA at 0, 0.2, 1 and 2 mM concentrations were prepared with deionized water. When dissolving the crystal salicylic acid, 1 mL 95% ethyl alcohol was used (Shettel and Balke, 1983) and solution pH was set to 4.5-5 using 0.1 N NaOH (Larque-Saavedra, 1978). Twenty four sterile jars of 150 mL were divided into four groups, with six jars in each; the first group of jars were put 100 mL 0 mM SA solution (control), the second group 100 mL 0.2 mM SA solution, the third group 100 mL 1 mM SA solution and the fourth group 100 mL 2 mM SA solution. Then each jar was closed with a sponge lid. The roots of the aforementioned one-week-old seedlings were washed with tap water and five homogeneous (~15 cm in length) seedlings were transplanted on the edges of each sponge lid. Thus, 30 homogeneous-looking seedlings were obtained in each group. After the markings aimed to facilitate measurement were made, the jars were put into growth cabins which had controlled conditions (intensity of light: natural light; temperature: 25±2°C, photoperiod: 16 h). In this way, the seedlings were exposed to SA concentrations for 3 days from their roots. Throughout this period, the jars were continuously checked and equal amounts of the corresponding solutions were added to the jars.

pH measurements were performed with JENWAY 3040 ion analysis using a pH-meter apparatus. Sartorius BL 120 fine scales were used in the weighing procedures. Absorbance measurements were carried out using an UVA-093001 Heλiosα spectrophotometer. Sigma and bovine serum albumin used in protein analysis (0.1 g mL^{-1}) were supplied by Sigma Diagnostics and other chemicals by Merck company. Seeds of radish used in our study were purchased from Istanbul seed bazaar. Their certificate number is K005 and harvest year is 2006.

Determination of fresh weight: In order to determine fresh weights, the preliminary weights of one-week-old seedlings were found and final weights of ten-day-old seedlings to which SA was applied from the root for 3 days were determined. The change in fresh weight was determined in g g^{-1} from the difference between the preliminary and final weight values. Ten seedlings were used in each weighing.

Determination of pigment amount: In order to find out the chlorophyll (a+b) amount, 0.5 g of fresh leaf obtained from ten-day-old seedlings which were applied SA from the root for 3 days was used in each group. For pigment analysis, the leaves were extracted (Witham *et al*., 1971) and the absorbances of these extracts were read against blind at 645 and 663 nm wavelengths. Quartz tubes of 1 cm^3 volume were employed in the absorbance determinations. Chlorophyll (a+b) amounts were calculated from the absorbance values obtained (Witham *et al*., 1971).

Protein extraction and determination: In order to establish the protein amount, fresh weights of ten-day-old

seedlings to which SA was applied from the root were determined without delay and protein analysis was conducted. Protein extraction was performed according to Larson and Beevers (1965) method described in Ross (1974). For this purpose, 1.4 g of plant (~10 seedlings) was used for each group. Following the extraction procedures described in the method, extracts were put into separate test tubes. Lowry method was employed to find out the total protein amount. The tubes containing the extracts were subjected to the procedures explained in the method (Lowry *et al*., 1951) and then, their absorbances were read against blind at 725 nm in the spectrometer, as described in the method. Standard curve prepared in advance from serum proteins of 0.25, 0.50, 0.75 and 1.00 mg mL^{-1} concentrations of the bovine serum albumin stock protein were utilized to calculate the protein amount (mg g^{-1} fresh weight).

Statistical analysis: All experiments were carried out in triplicate. The results were subjected to statistical analysis by calculating the standard deviation of the mean and by conducting a variance analysis (SPSS 10.0 Windows, Duncan and Kruskal-Wallis test).

RESULTS AND DISCUSSION

As it can be understood from the results in Table 1, application of 0.2 mM SA did not bring about a significant difference in fresh weight gain, relative to the control group ($p>0.01$). However, fresh weight gain in seedlings which were applied 1 mM SA and 2 mM SA was 30% and 63.42%, respectively, lower than that in the control seedlings and these values were found statistically significant ($p<0.01$).

As seen in Table 2, no significant difference was observed in the chlorophyll (a+b) amounts of radish seedlings to which 0.2 mM SA was applied ($p>0.01$), while chlorophyll (a+b) levels of radish seedlings to which 1 and 2 mM SA were applied measured 30.60 and 44.87% lower than those in the controls and this difference was found statistically significant ($p<0.01$).

Once again, 0.2 mM SA application did not create a significant difference in protein level of the seedlings ($p>0.05$), whereas 1 and 2 mM SA application reduced protein amounts by 27.20 and 40.82%, respectively, in comparison to the control seedlings ($p<0.05$) (Table 3).

It is highly difficult to properly discuss the results we obtained due to the lack of clear literature on this topic. The results brought about by high SA concentrations may be attributed to osmotic water correlations and stress due to a toxic effect. The decrease caused by high concentrations of SA in chlorophyll amount was claimed to have resulted from inhibition of chlorophyll biosynthesis, acceleration of chlorophyll destruction or both (Yang *et al*., 2002). It is known that SA and other salicylates have on the protein synthesis mechanism an effect that can be described as a control mechanism (Pennazio *et al*., 1983; Francesco *et al*., 1986). It can be suggested that the effects of SA on the enzyme level are mediated by plant hormones (Schneider and Whitman, 1974) and that high SA concentrations increase, rather than inhibit, ethylene biosynthesis (Romani *et al*., 1989; Huang *et al*., 1993).

In conclusion, all these physiological effects of SA, which is regarded a new growth regulator (Raskin, 1995; Losanka *et al*., 1997; Rajasekaran and Balke, 1998, 1999), should be considered at the hormonal dimension, despite lack of a proper base for discussion.

Table 1: Fresh weight gains determined in ten-day-old radish (*Raphanus sativus* L. cv. white) seedlings to which SA was applied for 3 days (g g^{-1})

Fresh weight alteration (g g^{-1})				
0 mM SA (Control) ($\bar{x} \pm S\bar{x}$)	0.2 mM SA ($\bar{x} \pm S\bar{x}$)	1 mM SA ($\bar{x} \pm S\bar{x}$)	2 mM SA ($\bar{x} \pm S\bar{x}$)	p-values
0.1123 ± 0.011^{ab}	0.1190 ± 0.004^{a}	0.07863 ± 0.009^{c}	0.04109 ± 0.011^{d}	<0.01

**: a,b,c,d: Differences between group means with different letter(s) in the same line are significant ($p<0.01$), n: 10

Table 2: Pigment amounts measured in the leaves of ten-day-old radish (*Raphanus sativus* L. cv. white) seedlings to which SA was applied for 3 days (mg g^{-1} fresh weight)

Cl (a + b) (mg g^{-1} fresh weight)				
0 mM SA (Control) ($\bar{x} \pm S\bar{x}$)	0.2 mM SA ($\bar{x} \pm S\bar{x}$)	1 mM SA ($\bar{x} \pm S\bar{x}$)	2 mM SA ($\bar{x} \pm S\bar{x}$)	p-values
1.493 ± 0.11^{a}	1.357 ± 0.12^{ab}	1.036 ± 0.10^{c}	0.823 ± 0.12^{d}	<0.01

**: a,b,c: Differences between group means with different letter(s) in the same line are significant ($p<0.01$)

Table 3: Protein amounts found in ten-day-old radish (*Raphanus sativus* L. cv. White) seedlings to which SA was applied for 3 days (g g^{-1} fresh weight)

Protein content (mg g^{-1} fresh weight)				
0 mM SA (Control) ($\bar{x} \pm S\bar{x}$)	0.2 mM SA ($\bar{x} \pm S\bar{x}$)	1 mM SA ($\bar{x} \pm S\bar{x}$)	2 mM SA ($\bar{x} \pm S\bar{x}$)	p-values
2.812 ± 0.14^{a}	2.559 ± 0.12^{ab}	2.047 ± 0.12^{b}	1.664 ± 0.11^{c}	$p<0.05$

*: a,b,c: Differences between group means with different letter(s) in the same line are significant ($p<0.05$)

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