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EFFECTS OF ACETYLSALICYLIC ACID ON FRESH WEIGHT PIGMENT AND PROTEIN CONTENT OF BEAN LEAF DISCS (*PHASEOLUS VULGARIS* L.)

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The effects of 100, 250, and 500 ppm acetylsalicylic acid solutions treatments on weight alteration, pigment and protein amounts in discs from the primary leaves of one month old bean (*Phaseolus vulgaris* L.) seedlings produced under greenhouse conditions are presented. The experiments show that: 100 ppm ASA had no significant influence ($P > 0.05$) but 250 and 500 ppm ASA caused an increase on weight loss ($P < 0.01$); ASA at higher concentrations (250 and 500 ppm), generally, caused a decrease on pigment amounts ($P < 0.05 – P < 0.01$) but 100 ppm ASA had no considerably significant influence on them ($P > 0.05$), none of the ASA treatments caused a statistically significant influence on carotenoid amount ($P > 0.05$); 100 and 250 ppm ASA treatments did not cause a significant influence on protein amount ($P > 0.05$), however 500 ppm ASA treatment caused an increase on protein injury ($P < 0.05$). Consequently, it is supposed that wet weight loss, pigment and protein injury have somewhat increased on leaf discs, depending on the toxic effect of high acetylsalicylic acid concentrations.

Keywords: Acetylsalicylic acid – toxic – leaf discs

INTRODUCTION

It is reported that salicylic acid (SA), whose commercial production form is acetylsalicylic acid (ASA), stimulates blooming [18, 23], has thermogenic effect [18], increases thermotolerance [11], is a natural herbicide [25], stimulates adventive rooting [13], reduces leaf abscission [9], provides resistance against pathogens [23], inhibits ethylene biosynthesis [7], alters quality and quantity of proteins [12] in some plants, and also increases the *in vivo* activity of nitrate reductase in maize seedlings [18].

ASA, as a phenolic compound, provides resistance to the plant against various stress factors [5, 8] and modifies the effects of abscisic acid [1] and cytokinins [19] during plant growth [20]. Although a secondary plant yield, SA is accepted as a significant plant hormone owing to its many common physiological effects [6, 16, 18].

It is known that cytokinins retard disintegration and increase synthesis of chlorophyll. Cytokinins have also some effects such as preventing plant tissue ageing and

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protein disintegration as well as increasing protein synthesis [3]. The decrease in the photosynthetic pigment content, observed in the leaf discs of *Vitis vulpina* under the salt stress, was suppressed by external 1 mmol zeatin application to the discs [15]. In the same research, it is observed that salt decreases enzyme activity in the discs. However, external zeatin application prevents this effect produced by salt.

Tissue culture, a direct measure of growth, is utilized in order to observe the growth, one of the basic steps of life. In recent studies made with corn (*Zea mays* L.) protoplast culture and carrot (*Daucus carota* L.) cell suspension culture, it is proven that low concentration of ASA is an efficient ethylene biosynthesis inhibitor [7, 22]. The relation between ethylene and the other plant growth regulators (abscisic acid, acetylsalicylic acid, benzyl adenine, gibberelline and indoleacetic acid) are quite controversial in the growth and development of tissue culture [6].

In the plant under stress, considerable metabolic changes take place and the plant ages earlier, generally. A decrease in synthesis or an increase in injury of protein as well as chlorosis and necrosis are noticeable indicators of ageing. Observing some effects of salicylic acid is easier on the plant being cut or tissues exposed to stress.

MATERIALS AND METHODS

Leaf discs were obtained from the primary leaves of one-month-old seedlings grown from bean seeds (*Phaseolus vulgaris* L. Strike) in sand culture not including mineral salts in greenhouse with a light approximately 2500 lux near the pot and 3000 lux 14–15 cm up near the pot, heat 25–27 °C during the day and 19–21 °C during the night, photoperiod 16 hours light, 8 hours dark (long day). Crystal acetylsalicylic acid was solved in 0.5 ml of 95% ethanol [25]. The pH of the all solutions including control (pure water) was adjusted to 4.5–5.0 with 0.05 M NaOH and HCl [14]. The pH measurements were performed with JENWAY 3040 ion analysis and pH-meter apparatuses. Sartorius BL 120S mark precision balance was used for weighing. Acetylsalicylic acid was provided from Sigma firm with 99.5% pureness in crystal condition.

Leaf discs, 2 cm in diameter, were cut from each side of the mid-veins of the uniform appearing primary leaves of seedlings [2]. For each one of control and ASA groups, 100, 250, 500 ppm, 5 pieces of Petri dishes, 11 cm in diameter, were used. Equal weighted 2-ply filter papers placed in Petri dishes were moistened with the group's own solution, 10 ml per Petri. The discs were placed to each Petri prepared in this way, in a form that, each Petri will have 6 discs whose first weights were determined and within such a plan that, which disc is where to be placed could be easily determined. Then, all the Petri dishes were covered and they were taken into the greenhouse for 96 hours. At the end of this period of time the surface waters of the discs were cleaned and their wet weights were determined. For all the parameters examined in the discs, the first hour of the first day's 16-hour light period was accepted as the beginning.

Wet weight determination

The Petri dishes were taken out of the greenhouse after 96 hours. The last wet weights of the discs were determined without any delay by using the differences between the first wet weight and the last wet weight values, the weight alteration was calculated as per cent [4].

Pigment analysis

In order to determine the amounts of chlorophyll-a, chlorophyll-b, carotenoid, total pigment I, and total pigment II the Petri dishes were taken out of the greenhouse after 96 hours. The last weights of the discs were determined without any delay and then pigment analysis was performed as done in the study by Witham et al. [26]. For this purpose, 1 g leaf disc tissue was used for each group. Besides, the absorbances of all these extracts were separately read in CE-5502 Scanning Double Beam UV Spectrophotometer in 440, 645, 652 and 663 nm wavelenghts against blind. Quartz tubes of 1 cm^3 were used in determining the absorbances. The amounts of chlorophyll-a, chlorophyll-b, carodenoid, total pigment I and total pigment II were calculated using these absorbance values [26].

Protein analysis

The standard curve

The method in the study by Ross [21] was used to determine total protein amount. For this purpose, at first, four solutions samples at concentrations of 0.25, 0.50, 0.75 and 1.00 mg ml^{-1} were prepared from the 0.1 g ml^{-1} cow serum albumin stock protein concentration, provided from Sigma Diagnostics. Then, the samples were processed as mentioned in the method and absorbance values were read against pure water in 725 nm with CE-5502 Scanning Double Beam UV Spectrophotometer. The standard curve was plotted using these absorbance values (Fig. 1).

Protein extraction and determination

Protein extraction was performed according to the method described in the study by Ross [21]. For this purpose, 1.5 g leaf disc tissue was used for each group. After the extraction processes mentioned in the method, the samples were put in an experiment tube and their absorbances were read against blind in 725 nm with CE-5502 Scanning Double Beam UV Spectrophotometer. The absorbance values read on spectrophotometer were marked on the graphic given in Fig. 1, and the correspondent

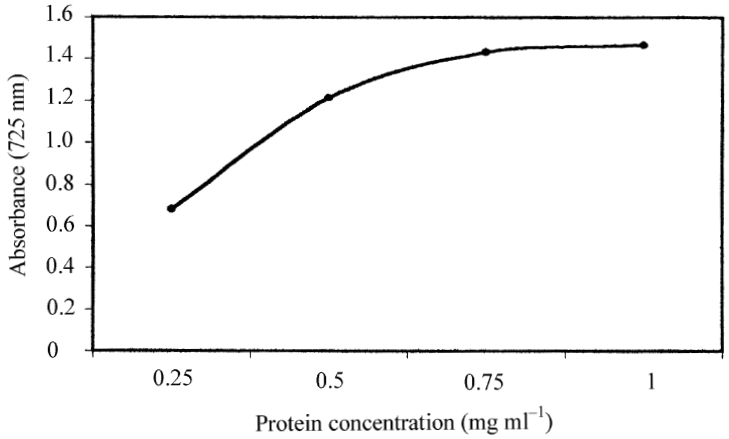

Fig. 1. Standard curve used for protein analysis of BSA protein (Sigma diagnostic)

protein concentrations were determined as wet weights mg g^{-1}. Then, protein amounts were calculated for per gram of the tissue in the 150 ml disc extract.

Statistical analyses

All the experiments were repeated for three times. The results were analyzed, statistically, by performing variance analysis (SPSS 10.0 Windows, Duncan and LSD test).

RESULTS

As to the control, the wet weight loss, for the groups treated with 250 and 500 ppm ASA were found as, 25.83% and 49.01% ($P < 0.01$), respectively (Table 1).

As to the control group: The chlorophyll-a amount in the bean discs was statistically identical ($P > 0.05$) for the group treated with 100 ppm ASA, lower as much as 27.44% and 47.85% ($P < 0.01$) for the groups treated with 250 and 500 ppm ASA,

Table 1
The effect of treatments of 100 ppm ASA, 250 ppm ASA and 500 ppm ASA on the fresh weight of bean (*Phaseolus vulgaris* L.) leaf discs

Wet weight alteration (%)			
Control	100 ppm ASA	250 ppm ASA	500 ppm ASA
10.14 ± 0.87	8.87 ± 0.34	7.52 ± 0.52	5.17 ± 0.87

Table 2
The effect of treatments of 100 ppm ASA, 250 ppm ASA and 500 ppm ASA on pigment concentrations of bean (*Phaseolus vulgaris* L.) leaf discs

Pigment variety	(mg g^{-1} wet weight)			
	Control	100 ppm ASA	250 ppm ASA	500 ppm ASA
Chlorophyll-a	1.607 ± 0.19	1.389 ± 0.11	1.166 ± 0.03	0.838 ± 0.02
Chlorophyll-b	0.531 ± 0.11	0.499 ± 0.02	0.430 ± 0.02	0.353 ± 0.02
Carotenoids	0.218 ± 0.02	0.191 ± 0.03	0.188 ± 0.02	0.189 ± 0.02
Total pigment I	2.138 ± 0.31	1.889 ± 0.17	1.592 ± 0.02	1.158 ± 0.02
Total pigment II	2.230 ± 0.33	1.963 ± 0.19	1.670 ± 0.03	1.273 ± 0.02

respectively. The chlorophyll-b amount, for the groups treated with 100 and 250 ppm ASA, was statistically identical ($P > 0.05$) and it was lower as much as 33.52% ($P < 0.05$), for the group treated with 500 ppm ASA. For the groups treated with 100, 250 and 500 ppm ASA, carotenoid amount was found statistically identical ($P > 0.05$). Total pigment I and II amounts were statistically identical ($P > 0.05$) for the group treated with 100 ppm ASA and lower as much as, 25.53%–25.14% and 45.83–44.55% ($P < 0.01$) for the groups treated with 250 and 500 ppm ASA, respectively (Table 2).

Table 3
The effect of treatments of 100 ppm ASA, 250 ppm ASA and 500 ppm ASA on protein content bean (*Phaseolus vulgaris* L.) leaf discs

Protein content (mg g^{-1} wet weight)			
Control	100 ppm ASA	250 ppm ASA	500 ppm ASA
2.225 ± 0.35	1.850 ± 0.2	1.725 ± 0.2	1.500 ± 0.25

The results, about the effects of the applications performed on bean discs over protein amount, are presented in Table 3. In comparison with the control group; in the groups treated with 100 and 250 ppm ASA, the protein amount was statistically identical ($P > 0.05$), in the group treated with 500 ppm ASA, however, it was found lower as much as 32.58% ($P < 0.05$).

DISCUSSION

It is difficult to validate the results of this study by comparing them with other published results because I have been unable to find any other data on this subject. The results, observed as weight decrement, may be originated from the deficiency of water absorption. Meanwhile, tissue injury depending on toxic effect of high ASA

concentrations is a significant factor. As a matter of fact, tissue softening with different proportions was observed macroscopically in these discs.

Also, it is known that SA and the other salicylates have an effect on protein synthesis metabolism, which can be described as control mechanism [10, 17]. It is claimed that the effects of ASA over enzyme level occur by means of plant hormones [24].

The decreases in pigment and protein amounts may be originated from the injuries being incited rather than the syntheses being prevented. As known, the levels of photosynthetic pigments and proteins are affected by multiple factors, either internal or environmental. Among the leading internal factors, the plant growth regulators come first. Mechanical effects, as respiration insufficiency, friction and damage besides natural processes, as ripeness and ageing, increase ethylene production in the leaves. It can be thought that high ASA concentrations promote ethylene biosynthesis rather than inhibit it [7]. As parallel to an increase at the concentration, any decrease in the pigment and protein amounts in the discs may depend either on the ABA level which increases or on cytokinine level which decreases because of toxic stress.

All these findings raise the possibility that SA, which is accepted as a new growth regulator [6, 16, 18], fulfils all these physiological and biochemical effects interacting with the other plant growth regulators.

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