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Effects of Salicylic Acid on Growth and Chlorophyll Destruction of Some Plant Tissues

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Abstract: How phenolic compounds act in the regulation of plant growth is controversial. The present study explores the effect of salicylic acid on some vegetal bioassays. Salicylic acid was not found to have any growth-stimulating effect on excised radish cotyledons. It was seen to exert a chlorophyll (a+b) loss delaying effect on leaf discs taken from radish cotyledons, while there was no such effect on leaf segments of barley. Likewise, it had no growth-stimulating effect on the elongation of wheat coleoptile segments. Marked inhibitive or toxic effects were observed in most of the investigated parameters for high salicylic acid concentrations.

Key words: Salicylic acid · S.A. Bioassays

Abbreviations: SA- salicylic acid · BA- benzyadenine · IAA- indolacetic acid · ABA- abcisic acid

INTRODUCTION

Tissue culture, a direct measure of growth, is used to observe growth, which is a major step in vitality. Many changes that arise in the process of growth can be observed in detail through the tissue culture. At present, plant production in closed systems where there are various food solutions has gained scientific acceptance [1]. These food solutions sometimes consist of phenolic compounds, which are secondary plant metabolites. Use of phenolic compounds in tissue culture technique is new and thus, the studies in this field are scarce.

Salicylic acid (SA) is an endogenous growth regulator of phenolic nature and was reported to take part in the regulation of the physiological process in plants [2]. Commercially manufactured form of salicylic acid is acetylsalicylic acid (ASA), which is known to hydrolyze almost entirely to its active ingredient in aqueous solutions [3]. This explains why the effects of ASA on plants were found similar to those of SA in some examinations [4-8].

Phenol derivatives were reported to affect enzymatic reactions from beginning to end by either modifying some mineral food and various organic substances in the medium or by influencing kinetin and IAA level [4].

It was reported in a study conducted in the protoplast culture of English ryegrass (*Lolium perenne*) that SA increased the expansion capacity of protoplast in the culture, but did not affect the regeneration (root, leaf, etc.) frequency [9]. Bourbouloux *et al* [10] who carried out a study in leaf discs of beet (*Beta vulgaris*) found that the increase in sugar and amino acid intake as a result of tissue aging was inhibited to a large extent by external SA administration (10-200 μM). In a study conducted on phenolic compound derivatives, it was established that majority of these had a betacyanin synthesis stimulating effect in foxtail (*Amaranthus caudatus* M.) cotyledons, growth enhancing effect in radish (*Raphanus sativus* L.) cotyledons and chlorophyll loss delaying effect in leaf segments of radish and barley [11]. In a study carried out on discs taken from primary leaves of one-month bean plants (*Phaseolus vulgaris* L. Strike), it was found that amounts of chlorophyll and protein were decreased, while fresh weight loss increased parallel to the rise in ASA concentration [12]. All these results demonstrate that SA must have an effect on cytokinin activity. It was reported that cytokinins stimulated chlorophyll synthesis [13] and that there was a correlation between endogenous cytokinin level and chlorophyll production capacity [14]. Cytokinins were also reported to stimulate chloroplast differentiation [15] and cell expansion [16] in cucumber cotyledons. Besides, it was found that cytokinins induced the molding of chloroplasts in radish cotyledons [17].

Similarly, Karanov *et al.* [11] reported that some phenolic derivatives inhibited elongation growth of some barley (*Hordeum vulgare* L.) coleoptile segments in high concentrations and stimulated the foregoing in low

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concentrations. This two-directional effect of phenolic derivatives on growth of coleoptile segment elongation may be explained by their having an effect on IAA metabolism [18,11]. It was reported that treatment with 0.05 mM SA increased the level of cell division in the apical meristem of the roots of wheat seedlings. SA application was also noted to cause accumulation of both ABA and IAA in wheat seedlings under stress. It was argued that SA treatments essentially reduced the change in the level of phytohormones (IAA and cytokinin) of wheat seedlings under stress [2].

Modification of plant growth and development by use of plant growth regulators has an ever-increasing importance in modern agricultural practice [19]. However, studies on this topic are still scarce. The controversial correlation between ethylene and growth regulators like ABA, ASA, BA, GA, IAA in the growth and development of tissue culture [20] makes it difficult to interpret studies in this field. Presence of new regulators imitating the effect of plant growth hormones is significant in controlling plant growth and development. Furthermore, discovery of new natural substances which have high a physiological activity in low concentrations is important to preserve the ecological balance. The present study aims to test some positive and negative effects of salicylic acid, which is commonly considered a hormone, in some specific bioassays.

MATERIALS AND METHODS

Preparation of Plant Materials and Solutions: In the present study, radish (*Raphanus sativus* L. White), barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L. Cumhuriyet) seeds were used as plant material. Test solutions of various concentrations (0 mM, 0.02 mM, 0.06 mM, 0.2 mM and 2 mM) of salicylic acid prepared with deionized water constituted the chemical substance. In order to dissolve crystal salicylic acid, 1 ml 95% ethanol was used [21] and the pH of all solutions was set to 5.5-6.5. Benzyladenine (BA) and indoleacetic acid (IAA) in concentrations of 0.01 mM were used as markers.

Biological Evaluations and Bioassay Procedures

Growth of Isolated Radish Cotyledons: Growth activity of excised radish cotyledons was tested by Letham [22]'s modified version. For this purpose, radish (*Raphanus sativus* L. White) seeds were wetted with tap water for 4 hours and then planted on wet cotton. They were left to germinate for 44 hours in the dark at 24°C. At the end of this period, homogenous seedlings were collected and their small cotyledon leaves were excised. These were placed on two-ply and equal weight filter papers moistened with 10 ml test solution each in Petri dishes of 11 cm. Their placement was planned so that their adaxial surfaces contacted the solution in the filter paper. After that, the Petri dishes were covered and left to wait for 72 hours under florescence light of 500 lux luminance (photoperiod: 12h L/ 12h D) at a medium temperature of 25-26°C. At the end of this period, cotyledons were blotted dry and weighed.

Destruction of Chlorophyll in the Leaf Segments: Chlorophyll retention was tested using Kende [23] 's modified version in radish and barley leaf segments. For this purpose, radish (*Raphanus sativus* L. White) seeds were wetted with tap water for 4 hours, planted on wet cotton and were left to germinate for 44 hours in darkness at 24°C. Then they were grown in a medium with natural light, 27/24 day/night temperature and 50% relative humidity, until they were two weeks old. At the end of this period the seedlings were selected on the basis of homogeneity and discs were taken from small cotyledon leaves of radish seedlings. Segments were also collected from primary leaves of two-week barley (*Hordeum vulgare* L.) seedlings grown in the same way. These were then placed on one-ply filter paper moistened with test solution in Petri dishes with a diameter of 9 cm. The radish was left to incubate for 24 hours and barley for 12 hours in darkness at 25°C. At the end of this period, chlorophyll was analyzed in leaf segments. For each replicate, 20 leaf segments were dried and their optical densities were measured [24].

Growth of Etiolated Wheat Segments: Wheat (*Triticum aestivum* L. Cumhuriyet) seeds, which were wetted with tap water for 5 hours, were vertically planted between folds of moistened filter paper within beakers. Then the beakers were kept in the dark at 24-25°C for 67 hours for germination. At the end of this period, coleoptiles which reached 3-3.5 cm in length were taken; 5 mm segments below the terminal 3 mm-part were excised under green light and let to float in deionized water for ½ hour in darkness at 23-24°C to eliminate endogenous hormones. Then 8 coleoptile segments each were put into black bottles containing 3 ml test solution and incubated for 20 hours in darkness at 26°C [25]. At the end of this period, coleoptile segments were removed from the bottles and their length was measured.

Statistical Analyses: All experiments were conducted in triplicates. Results were statistically analyzed by measuring the standard deviation of the mean and using variance analysis (SPSS 10.0 Windows, Duncan and Kruskal-Wallis test).

RESULTS

As Table 1 demonstrates, 0.06 mM SA administration did not produce a significant difference in the fresh weight of excised radish cotyledons, compared to the control ($p>0.05$). 0.2 mM and 2 mM SA administrations were found to cause a 36.55% and 66.56% less increase in fresh weight, relative to the control, respectively ($p<0.05$). 0.02 mM SA administration resulted in a statistically insignificant increase in excised radish cotyledons, when compared to the control ($p>0.05$) [9,11,16]. However, this increase was found very low, in comparison to the increase obtained with BA. When fresh weight changes in excised radish cotyledons are concerned, SA was observed to have an inhibitive effect, rather than a cytokinin-like activity.

Results pertinent to chlorophyll (a+b) retention in excised radish cotyledon discs and barley leaf segments are presented in Table 2. As it is understood from Table 2, the protection obtained with 0.02 mM SA administration in radish cotyledon discs relative to the control (5.49%) ($p<0.05$) [15,17,11], as well as the destruction caused by 0.2 mM and 2 mM SA (2.16%, 30.60%) ($p<0.05$) were found statistically significant. 0.02 mM and 0.06 mM SA administrations did not produce a statistically significant difference in barley leaf segments. 0.2 mM and 2 mM SA administrations were found to cause chlorophyll (a+b) destruction (12.88% and 21.29%), when compared to the control ($p<0.05$). The results indicate that, especially in high concentrations, SA has a toxic effect that brings about chlorophyll (a+b) destruction.

Table 3 presents the effects of SA test solutions on the elongation growth of wheat coleoptile segments. As seen in the table, the effect of 0.02 mM, 0.06 mM and 0.2 mM SA administrations on elongation growth of coleoptile segments was found statistically insignificant relative to the control. 2 mM SA administration was

Table 1: Effect of test solutions on growth of excised radish (*Raphanus sativus* L. White) cotyledons

Concetrations	0 mM SA	0.02 mM SA	0.06mM SA	0.2mM SA	2mM SA	0.01mM BA
Weight alteration						
(mg.mg^{-1}.fresh weight)	1.51±0.18^{a}	1.62±0.21^{a}	1.41±0.17^{a}	1.15±0.20^{b}	0.85±0.23^{c}	2.16±0.20^{d}
Weight alteration (%)	151.99±18.11	162.57±21.06	141.37±17.47	115.44±20.07	85.43±23.25	216.60±20.59

a,b,c,d: Differences between group means with different letters in the same line are significant ($p<0.05$), n: 10, control (0 mM SA) : onset weight:5.02 mg, final weight: 12.65mg

Table 2: Effect of test solutions on chlorophyll destruction in segments excised from radish (*Raphanus sativus* L. White) cotyledons and barley (*Hordeum vulgare* L.) leaves

Concetrations	0 mM SA	0.02 mM SA	0.06 mM SA	0.2 mM SA	2 mM SA	0.01 mM BA
Radish Chlorophyll (a+b) amounts (mg.g^{-1}.fresh weight)	0.782±0.02^{a}	0.825±0.02^{b}	0.802±0.03^{a}	0.773±0.02^{c}	0.542±0.03^{d}	1.058±0.03^{e}
% to the control (0 mM SA)	100±0,00	105.49±3.14	102.55±3.06	98.84±2.23	69.30±3.47	135.29±5.80
BarleyChlorophyll (a+b) amounts (mg .g^{-1}.fresh weight)	0.893±0.04^{a}	0.827±0.03^{a}	0.810±0.05^{a}	0.748±0.03^{b}	0.702±0.03^{b}	1.106±0.04^{c}
% to the control (0 mM SA)	100±0.00	92.60±8.01	90.70±9.50	87.12±8.75	78.61±7.06	123.85±10.02

a,b,c,d,e: Differences between group means with different letters in the same line are significant ($p<0.05$) control (0 mM SA): radish Chl (a+b): 0.782 (100%); barley Chl (a+b): 0.893 (100%)

Table 3: Effect of test solutions on growth of wheat (*Triticum aestivum* L. Cumhuriyet) coleoptile segments

Concetrations	0 mM SA	0.02 mM SA	0.06 mM SA	0.2 mM SA	2 mM SA	0.01 mM BA
Elongation (mm)	2.1±0.21^{a}	2.5±0.18^{a}	2.2±0.16^{a}	1.9±0.20^{a}	1.6±0.15^{b}	3.3±0.23^{c}
% to the control (0 mM SA)	100±0.00	127.77±32.01	116.66±24.07	105.55±10.06	88.88±11.42	194.44±23.55

a,b,c: Differences between group means with different letters in the same line are significant ($p<0.05$), n: 8, control (0 mM SA): onset height: 5 mm, final height: 7.1 mm (100%)

observed to lead to 11.12% less elongation growth in coleoptile segments, compared to the control ($p<0.05$). Tissue macerations were seen especially in high SA concentrations.

DISCUSSION

Phytohormones are known to play a key role in the regulation of plant growth. It was reported that aging in excised rice leaves was characterized by a decline in chlorophyll and protein content [26]. As it is known, when exogenously administered, ABA and cytokinins start and delay leaf aging, respectively [27]. In the present study we observed that high concentrations of SA administrations had a marked inhibitive effect on excised radish cotyledons and excised wheat coleoptile segments. The inhibitive effect found on excised wheat coleoptile segments suggests that SA incites IAA- oxidation [18]. Similarly, high SA concentrations were found to spur chlorophyll (a+b) destruction in both radish leaf discs and barley leaf segments. Application of 0.02 mM SA prevented chlorophyll destruction in radish leaf discs. All these require SA to have a bidirectional effect on cytokinin metabolism. However, current interpretations cannot find enough support. It is highly difficult to discuss these results in a meaningful way, as there is no clear literature on this subject. Use of some organic acids imitating the effect of plant growth hormones in such studies would enable a better understanding and interpretation of research results.

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