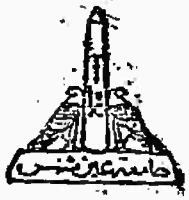

حولية كلية البنات

جامعة عين شمس

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العدد الثامن عشر ١٠ يناير ١٩٩٣

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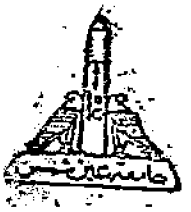

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Effect of Salicylic Acid and Gallic Acid on the Growth Responses and Physiological Changes of *Vicia faba* (cv. Giza 402) Seedlings.

I. Changes in growth responses, carbohydrate contents and activities of certain enzymes

By

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Abstract

The influence of two concentrations (1and 5mM) of salicylic acid (SA) and gallic acid (GA) on the changes of growth rate, carbohydrate contents and the activity levels of certain enzymes in *Vicia faba* (cv. Giza 402) seedlings were examined in the present study.

Treatment with SA and GA at 1 and 5mM inhibited the % of seed germination and supressed the rate of seedling growth (expressed as mean length of shoot and root and mean number and length of the lateral roots) except GA at 1mM which stimulated the seedling growth. GA at 1mM increased the activity level of catalase while it decreased AA-oxidase activity as compared with the untreated seedling. On the other hand, treatment with SA (1 and 5mM) and GA (5mM) declined catalase while they increased AA. oxidase activities below and over those of the untreated seedlings.

It was found that, the activities of polyphenol oxidase and invertase enzymes and the contents of reducing sugars (except at 1 and 2 days) polysaccharides and total carbohydrates were lower in seedlings treated with SA and GA than in the control ones. On the other hand, seedlings imposed to SA and GA at 1 and 5 mM, caused an elevation of both sucrose contents (except for seedlings grown with SA5) and α and β– amylase activity over those estimated in the control seedlings.

Key words: *Vicia faba*, Gallic acid at 1mM : GA1, Gallic acid at 5 mM: GA5, Salicylic acid at 1 mM: SA1, Salicylic acid at 5 mM: SA5.

Introduction

Many phenolic compounds, the most commonly identified as phytotoxins produced by higher plants (Rice, 1984) are widely spread in soil (Wang *et al.*, 1967). Whitehead

[illegible] found that benzoic and cinnamic acid derivatives are present in the soil solution at the concentration ranges of 0.01 to 0.1mM. These allelochemicals are released into the soil system by plant decomposition, leaching from leaves and root exudation (Guenzi and McCalla, 1966; Rovira, 1969 and Tukey, 1970).

Phenolic compounds have been shown to be of importance in the regulation of plant growth and metabolism and are no longer considered to be passive by-products (Jain and Srivastava, 1981). Ferulic acid (FA) alters seed germination (Leather and Einhellig, 1984), inhibits radicle growth (Blum *et al.*, 1984) and decreases leaf expansion, leaf production, dry weight accumulation (Patterson, 1981; Blum and Dalton, 1985 and Blum *et al.* 1985 a,b) photosynthetic rates (Patterson, 1981) and leaf chlorophyll content (Einhellig and Rasmussen, 1979 and Patterson, 1981). The magnitude of these responses varies with the concentration of ferulic acid (Einhellig and Eckrich, 1984).

Decreased leaf water potential (ψ_L), shoot turgor pressure (ψ_T) and osmotic potential (ψ_π) have been observed in soybean and sorghum seedlings after treatment with 250 to 1000uM FA for one day (Patterson, 1981 and Einhellig *et al.*, 1985). Transpiration and water utilization in cucumber seedlings decreased after 2 days of treatment with 500uM FA (Blum *et al.*, 1985 b). However Blum *et al.* (1985 b) noted that cucumber seedlings wilted within 2 h of treatment with 500uM FA but visibly recovered within 24 h. They suggested that FA temporarly modified water uptake by the roots, which reduced the inward diffusion of water needed for cell and leaf expansion.

Ahmed (1987) found that all doses of Na-salicylate caused significant decreases in reducing sugars , sucrose and polysaccharide contents at the early stages of leaf growth of wheat plant.

The present study deals with the effect of monophenolic acids (salicylic acid, SA) and polyphenolic acids (gallic acid, GA) on the growth, carbohydrate metabolism and activities of certain oxidative and hydrolytic enzymes during seed germination and early seedling growth of *Vicia faba* cv. Giza 402.

Materials and Methods

Growth of the plants.

Seeds of *Vicia faba* cv. Giza 402 were surface sterilized with 0.1 % $HgCl_2$ for 10 min and then washed thoroughly with sterile water. The seeds were germinated in plastic pots (15 cm in diameter and 11 cm in depth) on Whatman filter paper no. 45 under the following conditions: relative humidity of 60-65 %, day length of 12 h and day/night air temperature: 22/18°C. Ten seeds were planted in each pot and all pots were arranged into 5 groups each of 8 pots, then each group recieved 15ml of the following solutions.

1- Distilled water to represent the untreated plants.

2- Salicylic acid at the concentrations of 1 and 5mM.

3- Gallic acid at the concentrations of 1 and 5mM.

The solution of each treatment (pH7.8) was daily renewed and at intervals of 1,2,7 and 14 days, germinated seeds or seedlings of only 2 pots were harvested per treatment.

The percentage of germination and certain morphological measurements were taken and statistically analysed using the least significant difference (LSD) at 1% and 5% levels of probability (Snedecor and Cochran,1967).

Methods of analysis

Estimation of carbohydrates

The different carbohydrate fractions were determined in the oven dried plant tissues. The methods of extraction and clarification were similar to those adopted by Said and Naguib (1964). The direct reducing sugars were determined following the procedure of Somogyi (1937) as described by Younis (1963). The total reducing sugars were estimated after the hydrolysis of the sucrose by invertase. The sucrose content was calculated from the difference between the total reducing sugars and the direct reducing sugars. Polysaccharides were estimated in the dry residue left after extraction of soluble sugars by the method adopted by Naguib (1963).

Enzyme assays

Crude extracts were prepared by homogenizing 1-5g fresh matter with 30 cm^3 Tris-HCl buffer pH 7.4 (Guerrier and Strullu, 1990). The homogenates were centrifuged at 7000g for 30 min and the supernatants were directly used for the enzyme assays.

Activities of ascorbic acid oxidase (AA-oxidase), polyphenol oxidase and catalase were assayed according to the methods described by Mukherjee and Choudhuri (1981), Kar and Mishra (1976) and Biswas and Choudhuri (1976) respectively. Activities of AA-oxidase and polyphenol oxidase were expressed as the change in the optical density g^{-1} fresh weight h^{-1}. Catalase activity was expressed as u mole H_2O_2 destroyed g^{-1} fresh weight h^{-1}.

Activities of α and β amylases were assayed according to the methods described by Davis (1977) and Malik and Singh (1980) respectively- Activity of α- amylase was expressed as the decrease in optical density per unit time. The β-amylase activity, on the other hand, was expressed as ug maltose released from starch by the enzyme g^{-1} fresh weight h^{-1}. Invertase activity was assayed according to the method used by Russell and Jimmy (1980) with some modifications. The modifications were that the reducing sugars liberated from sucrose were determined by the method of Somogyi (1937). Invertase activity was expressed as mg reducing sugars released by the enzyme g^{-1} fresh weight h^{-1}.

Results

Effect of SA and GA on seed germination and seedling growth of *Vicia faba*.

Results presented in table 1 show that SA and GA at their used concentrations (1 and 5 mM) variably affected the % of seed germination of *Vicia faba* plants (24 h after treatment) so that high significant inhibition, complete inhibition, high significant stimulation and significant inhibition were observed at SA1, SA5, GA1, and GA5 respectively as compared to the control results. *Vicia faba* seeds imposed to SA1, SA5, GA1 and GA5 germinated at the % of 73.3, 60., 100 and 66.7. successively after 2 days which were highly significantly decreased (except GA1) than the contral results.

The protrusion of the plumule was completely inhibited in seeds imposed to while SA1, GA1 and GA5 highly significantly stimulated the plumule length at ag days above the untreated seeds. The radicle length was found to be significant highly significantly decreased at SA1 and SA5while GA1 and GA5 highly signif and significantly increased this criterion if being compared with that of the c seedlings after 7 days. All treatments adopted highly signilicantly depressed the length of both shoots and roots below the control seedlings after 14 days wi exception that GA1 highly significantly increased the same criteria. At the same a days), all treatments used (except SA1) either highly significantly decreased the n of lateral roots or highly significantly stimulated (except GA5) the mean length of roots below and above those of the untreated seedlings respectively.

Effect of SA and GA on the carbohydrate contents:

Reducing sugars: The contents of reducing sugars determined in *Vici* seedlings imposed to SA_1,SA5 GA1 and GA5 at 1 day and SA_1 and SA_5 at 2 day obviously elevated over that determined in the corresponding control. On the othe the same contents were found to be markedly declined in *Vicia faba* seedlings impo GA_1 and GA5 at 2 days and SA1, SA5, GA1 and GA5 at 7 and 14 days below th the controls. Among the used phenolic acid treatments, SA5 and GA1 gave the h and the lowest values of reducing sugars respectively throughout the experimental (table 2).

Sucrose: Table 2 shows that treatment with GA1, GA5 and SA1 obviously el the level of sucrose while SA5 treatment lowered this level above and below the st level determined in water - grown plants at all plant ages. The highest value of su was determined in plants imposed to 1mM gallic acid.

Polysaccharides: The polysaccharides contents determined in variously t and untreated *Vicia faba* seedlings were progressively and markedly decreased acr the plant age. Moreover this fraction was found to be lower in all treatments emp than those of the untreated plants. The order of decreases were as follows: Co SA5> SA1> GA5> GA1.

Total carbohydrates: The values of total carbohydrates estimated in *Vicia faba* plants treated with SA1,SA5,GA1 and GA5 were found, in most cases, to be lower than those detected in the untreated plants. The sequence of decrease were shown to be: control> SA1> SA5> GA1> GA5 except in germinated seeds of 1 day-old.

Effect of SA and GA on the activities of invertase, α and β- amylases:

The relative changes in the activities of invertase, α and β-amylases are shown in table 3. As compared to the untreated plants the invertase activity of *Vicia faba* plants was obviously decreased in responsc to the phenolic acids treatments, at all ages. The sequence of decreases were found to be: control > SA1> SA5> GA1> GA5.

In contrast, the activities of α and β-amylases were markedly elevated above the untreated plants throughout the experimental period. The order of increases were as follows: GA1> GA5> SA1> SA5> control.

Effect of SA and GA on the activities of certain oxidative enzymes.

Throughout the experimental period, GA1 markedly increased the activity of catalase enzyme above that of the control, whereas SA1, SA5 and GA5 markedly decreased the catalase activity below that of the control plants. The magnitude of decrease was most pronounced in plants imposed to SA5 (table 4). The sequence of increases in the catalase activity were: SA5> GA5> SA1> control .

Table 4 shows that SA1, SA5, GA1 and GA5 markedly declined the activity levels of polyphenol oxidase below those of the corresponding controls at the all plant ages. The sequence of decreases were as follows: control > SA1> SA5> GA1> GA5.

The activity levels of AA- oxidase detected in *Vicia faba* plants treated with SA1, SA5 and GA5 were found to be markedely elevated above those of the corresponding controls, while the activity of the same enzyme delected in plants imposed to GA1 was found to be lower than that detected in the control plants (table4).

Discussion

Phenolic acids at the concentrations used in this study significantly influen germination and growth of *Vicia faba* plants. SA and GA mostly inhibited se germination, SA significantly supressed the mean length of shoot and root ar significantly increased the number and mean length of lateral roots at the latest stage seedling growth. GA at the low concentration employed, generally increased the pla growth while its high concentration significantly suppressed the rate of the plant grow Phenolic compounds have been shown to be of importance in the regulation of pla gorwth and metabolism. It was noted that a low concentration of SA increased the grow of maize seedlings, while the higher concentration inhibited it (Jain and Srivastav 1981). Ferulic acid (FA) was found to alter seed germination (Leather and Einhellig 1984), inhibits radicle growth (Blum *et al.*, 1984) and decreases leaf expansion and le production (Patterson, 1981; Blum and Dalton, 1985). The magnitude of these respons varies with concentration of FA (Einhellig and Eckrich, 1984). Several lines of eviden point to water stress as an important factor in the observed responses to FA. Transpiration and water utilization in cucumber seedlings decreased after 2 days of treatment with 500uM FA and it modified water uptake by the roots which, in turn, reduced the inward diffusion of water needed for cell and leaf expansion (Blum *et a* 1985 a,b and Booker *et al.*, 1992).

Although the role of phenolic substances as a plant morphogentic regulators is wel known, there has been little study of the mechanism at the molecular level. The prese study proved that the phenolics at their high concentration used, decreased the activi levels of catalase and polyphenol oxidase while increased the activity level of AA-oxidase. In this connection, Hassanein *et al.* (1987 b) found that treating sorghu grains with coumarin resulted in decreased activities of catalase and peroxidase enzyme Positive correlation occurs between phenolic acids treatment and water stress in th treated plants (Patterson, 1981; Einhellig *et al.*, 1985 and Booker *et al.*, 1992). In th respect, Dwivedi *et al.* (1979), Mukherjee and Choudhuri (1981) and Hassanein an El-Telwany (1989) observed that water stress lowered the activity of catalase an AA-oxidase in the studied plants.

Thus the significant changes in the studied oxidative enzymes, in the present work, in response to seed treatment with phenolic acids used, may cause the accumulation of certain toxic substances that affect the metabolic pathways and consequently the rate of plant growth. The quinones formed by the oxidation of phenolic substances are quite toxic to the plant cell enzymes (Mayer and Harel, 1979), and the reduction in the activity of catalase may result in accumulated toxic levels of hydrogen peroxide which may be involved in the suppressed growth rate of *Vicia faba* plants imposed to phenolic acids in the present study.

Treating seeds and seedlings of *Vicia faba* with SA and GA gave rise, in general, to decreased levels of reducing sugars, polysaccharides and total carbohydrates while they caused general increases in the sucrose contents below and above those of the untreated plants respectively.

It may be stated that the effect of phenolic compounds on the metabolism varies with the plant, stage of plant growth, the organ of the plant and the doses of phenolics used. Ahmed (1987) found that all doses of Na-salicylate caused significant decreases in reducing sugars, sucrose, and polysaccharide contents at the early stages of leaf growth of wheat plant. However, Hassanein *et al.* (1987 a) found that no obvious changes in polysaccharides and reducing sugars contents and marked decreases in sucrose content were observed in coumarin treated sorghum grains as compared with those of the untreated ones.

Our results also showed that, SA and GA decreased the activity of invertase while they increased the activities of α and β-amylases. In this respect, Hassanein *et al.* (1987 a) found that coumarin inhibited markedly the activities of amylases and invertase in sorghum grains. The relative amounts of sucrose in plants may be determined by the relative activities of both sucrose synthetase and invertase enzymes. On the other hand, there are more or less possitive relationship between the amounts of each of sucrose and reducing sugars and the activity of invertase since the reducing sugars may be used as respiratory substrates and/or in building the amino acids, amides and proteins (Abdalla *et al.*, under press). The changes in α and β-amylases activities of the treated and untreated

seed. and seedlings of *Vicia faba* are observed to coincide with the correspc changes in the polysaccharides level. These results are supported by the results ob by Hassanein *et al.* (1987 a). In addition, the decline in polysaccharides and conseq the total carbohydrate contents particularly in seedlings of 7 and 14 days old m attributed in part to the increased activities of α-and β-amylases and in part to the inh photosynthetic rate caused by phenolics treatments. In this respect, FA trea decreased leaf production and dry weight accumulation (Patterson, 1981; Blum Dalton, 1985 and Blum *et al.*, 1985 a,b), leaf chlorophyll content (Einhelli Rasmussen, 1979) and photosynthetic rates (Patterson, 1981).

Conclusion: It may be stated that treatment with SA and GA, decreased the p germination and the growth rate of *Vicia faba* seedlings except at 1mM gallic acid. S increased the activity of catalase while it decreased AA-oxidase activity that may role in the obserbed stimulated plant growth. On the other hand, the declined activ catalase and the increased one of polyphenol oxidase especially at SA1, SA5 and may be participated in the inhibition of seed germination and seedling grow addition, treating seeds and seedlings of *Vicia faba* with the used phenolic acids i increased activities of α and β- amylases and sucrose content, while they decreased of invertase activity, reducing sugars, polysaccharides and total carbohydrate conten

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Table (1) Effect of salicylic acid and gallic acid on the % of germination, shoot and root length, mean number and length of lateral roots of *Vicia faba* seedlings. Each value is a mean of 8 replicates

Concentration (m M)	Days after treatment: 1	2	7	14			
	% of germination		Length of plumule	Length of radicle	M. L. of shoot	M. L. of root	M. No. of lateral roots	M. L. of lateral roots
Control (H_2O)	33.3	100	0.2	2.4	10.8	12.3	19	1.9
SA1	13.3-SH	73.3-SH	0.7 + SH	2.0 - S	7.0 - SH	5.3 - SH	21 + SH	4.8 + SH
SA5	0.0	60.0-SH	0.0	0.4 - SH	4.3 - SH	3.1 - SH	2 - SH	1.7 + SH
GA1	46.7+SH	100 NS	1.5 + SH	3.1 + SH	14.6 + SH	14.5 + SH	11 - SH	4.1 + SH
GA5	26.7- S	66.7-SH	0.5 + SH	2.8 + S	6.1 - SH	6.3 - SH	7 - SH	1.1 - SH
L. S. D at 5 %	4.8	5.1	0.03	0.4	0.75	0.86	0.93	0.12
L. S. D at 1 %	7.4	7.9	0.05	0.6	1.04	1.08	1.18	0.18

HS = high significant S = significant NS = non significant

ML = mean length M.No. = mean number

Table (2) Effect of salicylic acid and gallic acid on the carbohydrate contents during seed germination and early seedling growth of *Vicia faba* . Values listed are the average of triplicate determinations and expressed as mg glulcose equivalent g^{-1} dry weight (± sd) .

Age/ day	Concentration (m M)	Reducing sugars	Sucrose	Polysaccharides	Total carbohydrates
1	Control (H_2O)	13.1 ± 3.5	17.5 ± 2.6	88.7 ± 10.5	119.3 ± 20.3
	SA1	20.8 ± 3.8	30.1 ± 5.3	77.0 ± 8.7	127.9 ± 15.8
	SA5	26.9 ± 4.5	14.7 ± 2.5	80.6 ± 8.8	122.2 ± 15.8
	GA1	14.7 ± 3.3	35.6 ± 4.7	67.8 ± 5.9	118.1 ± 15.6
	GA5	16.8 ± 2.8	22.4 ± 3.6	73.3 ± 5.6	112.5 ± 10.5
2	Control (H_2O)	14.7 ± 4.1	11.9 ± 2.8	84.6 ± 8.3	111.2 ± 15.3
	SA1	16.8 ± 3.2	17.5 ± 2.2	71.8 ± 7.8	106.1 ± 10.4
	SA5	18.9 ± 3.5	8.8 ± 3.5	74.8 ± 6.3	102.5 ± 8.9
	GA1	11.2 ± 3.2	25.9 ± 5.4	61.2 ± 7.1	98.3 ± 12.1
	GA5	13.3 ± 2.2	15.2 ± 2.4	66.5 ± 6.2	95.0 ± 10.3
7	Control (H_2O)	20.3 ± 5.3	14.7 ± 3.3	79.2 ± 9.4	114.2 ± 18.6
	SA1	15.4 ± 4.4	19.3 ± 3.2	66.5 ± 5.8	101.2 ± 14.1
	SA5	16.8 ± 3.3	12.6 ± 1.8	69.6 ± 7.4	99.0 ± 8.6
	GA1	9.9 ± 4.5	27.3 ± 4.2	50.0 ± 5.5	87.2 ± 8.1
	GA5	12.6 ± 3.9	16.8 ± 2.0	55.8 ± 5.5	85.2 ± 6.4
14	Control (H_2O)	17.5 ± 5.6	17.5 ± 5.3	68.8 ± 10.3	103.8 ± 11.1
	-SA1	12.6 ± 3.6	21.4 ± 4.1	62.5 ± 7.5	96.5 ± 8.3
	SA5	14.7 ± 3.0	15.4 ± 2.6	65.2 ± 7.3	95.3 ± 6.8
	GA1	7.8 ± 3.1	30.1 ± 6.1	46.4 ± 3.8	84.3 ± 6.4
	GA5	10.9 ± 2.8	19.3 ± 3.6	50.0 ± 3.2	80.2 ± 5.9

Table (3) Effect of salicylic acid and gallic acid on the activities of invertase, -and - amylases during seed germination and early seedling growth of *Vicia faba* . Values listed are the average of triplicate determinations (± sd) .

Age/ day	Concentration (m M)	Invertase	- amylase as a decrease in optical density per unit time	- amylase as ug maltose released from starch by the enzyme g f.w.h
1	Control (H_2O)	6.6 ± 1.3	0.45 ± 0.11	10 ± 3
	SA1	3.7 ± 0.6	0.39 ± 0.12	20 ± 4
	SA5	2.8 ± 0.5	0.43 ± 0.10	15 ± 3
	GA1	2.0 ± 0.5	0.33 ± 0.15	30 ± 5
	GA5	1.1 ± 0.5	0.35 ± 0.13	25 ± 4
2	Control (H_2O)	4.5 ± 0.8	0.41 ± 0.16	15 ± 3
	SA1	3.3 ± 0.6	0.34 ± 0.11	25 ± 4
	SA5	2.4 ± 0.4	0.38 ± 0.06	20 ± 4
	GA1	1.5 ± 0.3	0.28 ± 0.05	40 ± 6
	GA5	0.5 ± 0.1	0.31 ± 0.08	30 ± 7
7	Control (H_2O)	8.3 ± 1.1	0.34 ± 0.10	30 ± 5
	SA1	7.5 ± 0.8	0.26 ± 0.03	45 ± 7
	SA5	6.3 ± 0.8	0.39 ± 0.05	35 ± 3
	GA1	4.8 ± 0.5	0.20 ± 0.03	70 ± 8
	GA5	3.4 ± 0.5	0.23 ± 0.03	55 ± 6
14	Control (H_2O)	11.8 ± 1.3	0.44 ± 0.10	40 ± 5
	SA1	9.2 ± 1.5	0.31 ± 0.06	50 ± 5
	SA5	8.3 ± 0.8	0.39 ± 0.05	45 ± 3
	GA1	5.0 ± 0.3	0.25 ± 0.03	80 ± 7
	GA5	4.2 ± 0.7	0.28 ± 0.03	75 ± 7

Table (4) Effect of salicylic acid and gallic acid on the activities of some oxidative enzymes during seed germination and early seedling growth of *Vicia faba*. Values listed are the average of triplicate determinations (± sd).

Age/ day	Concentration m M	Catalase	Polyphenol-oxidase	AA - oxidase
1	Control (H_2O)	336 ± 33	58 ± 8	6.0 ± 1.3
	SA1	192 ± 10	29 ± 5	6.5 ± 1.0
	SA5	96 ± 10	15 ± 5	8.0 ± 1.4
	GA1	480 ± 25	12 ± 2	5.1 ± 0.6
	GA5	168 ± 15	9 ± 2	7.0 ± 0.8
2	Control (H_2O)	432 ± 20	25 ± 6	6.3 ± 0.9
	SA1	288 ± 20	12 ± 3	7.5 ± 0.6
	SA5	192 ± 15	10 ± 3	8.6 ± 0.8
	GA1	528 ± 26	8 ± 2	5.5 ± 0.6
	GA5	240 ± 28	5 ± 1	7.8 ± 1.2
7	Control (H_2O)	480 ± 26	48 ± 5	4.8 ± 0.3
	SA1	432 ± 22	42 ± 5	5.8 ± 0.3
	SA5	288 ± 16	39 ± 3	6.6 ± 0.8
	GA1	624 ± 19	31 ± 3	2.9 ± 0.2
	GA5	336 ± 18	25 ± 3	6.2 ± 0.5
14	Control (H_2O)	920 ± 36	60 ± 6	3.5 ± 1.1
	SA1	720 ± 33	48 ± 5	4.1 ± 0.7
	SA5	336 ± 19	43 ± 3	5.5 ± 0.7
	GA1	1392 ± 46	36 ± 4	2.3 ± 0.4
	GA5	528 ± 21	29 ± 4	4.8 ± 0.3