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Differential Effect of Jasmonic Acid on the Defense of Faba Bean Against *Fusarium* Wilt: Modulation of other Phytohormones and Simple Phenols

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ABSTRACT

Two concentrations (5 mM & 10 mM) of jasmonic acid (JA) were used as soaking treatments of faba bean seeds. The fungicide Rhizolex-T was also similarly applied at a recommended potent rate (500ppm). Sowing was then carried out in normal as well as infected soil with *Fusarium* (*Fusarium oxysporium* f.sp. *fabae*). Faba bean plants (30-day-old) showed a potential resistance against the pathogen as a result of treatment with 10 mM JA, which approximately mimicked that of the fungicide, whereby a partial efficiency was achieved by 5 mM JA. Differential effects were evident by the two used concentrations of JA with reversed patterns under the influence of infection and in its absence. Thus, in normal soil 5 mM JA induced an evidently stimulatory rate of growth, but in the infected plants, a reverse situation was shown where 10 mM JA was markedly more effective. Both cases were underlined by concomitant changes in the levels of abscisic acid (ABA), gibberellic acid (GA3), indoleacetic acid (IAA), and the cytokinins zeatin and bezyladenine. The modulations in ABA led to assume a cross talk with JA, where enhanced levels were evident in response to JA in the non-infected plants, particularly in root. This increase was directly proportional with the applied concentration of JA. Roots of the plants treated with 10 mM JA, which performed an efficient defense, showed a dramatic drop in ABA content with several folds increase in the GA3/ABA ratio. The changes in other phytohormones and in phenolic contents with different treatments were also interpreted.

Key Words: Faba bean; Fusarium wilt; Jasmonic acid; Phenols; Phytohormones

INTRODUCTION

Jasmonic acid (JA) and its methyl derivative (MeJA) represents a class of plant growth regulators that plays pervasive roles in several aspects of plant development (Crozier *et al*., 2000). Among these are, seed germination, pollen development, responses to mechanical and insect wounding, pathogen infection, and drought stress (for further information see reviews by Hildebrand *et al*., 1998 and Schaller, 2001). Recent molecular genetic studies have confirmed the involvement of JA both in developmental (Sanders *et al*., 2000; Stintzi & Browse, 2000; He *et al*., 2002) and defense- related processes (Hammond-Kosack & Jones, 2000; Ryan, 2000). Biosynthetic pathways starting with α-linoleinic acid has been elucidated (Schaller, 2001). In this respect, two pathways may exist for JA biosynthesis, a chloroplast-localized (Creelman & Mullet, 1995) and a cytoplasmic (Wang *et al*., 1999) pathway. The biosynthesis of JA is regulatory adjusted in plant tissues and it is subject to inductive control by various elicitors including wounding (Wang *et al*., 2000) and effect of pathogens (Hammond-Kosack & Jones, 2000).

Upon pathogen attack, infected plant cells generate signaling molecules to initiate defense mechanisms in surrounding cells to limit pathogen spread (Baker *et al*., 1997). The role of JA, ethylene, and salicylic acid (SA) in this process is well documented at the molecular level, where such information is still elusive with other plant hormones (Audenaert *et al*., 2002). The same authors revealed that involvement of Abscisic acid (ABA) is based on indirect observations. For example, increased endogenous ABA levels were recorded in response to infection with viruses, bacteria and fungi (Kettner & Dőrffling, 1995). But, it is generally found that application of exogenous ABA increases the susceptibility of plants to fungal pathogens (McDonald & Cahill, 1999). This was linked in some cases with suppressive effects of ABA on phenylalanine ammonia lyase (Fraser, 1991). It should also be added that ABA is involved in the wound response activated upon insect feeding (Birkenmeier & Ryan, 1998). In this respect, there are sound arguments on whether plant responses provoked by the invasion of a pathogenic organism differ or not, from those induced by wounding (Hammond-Kosack & Jones, 2000). In contrast, there have been few reports of beneficial effects of ABA on fungal infections or related situations. In this connection, a fungicide, triadimefon, was found to operate via its effects on ABA acid concentration in treated bean plants (Fraser, 1991). Related disciplines did not refer to direct cross-talk events between JA and other phytohormones (Światek *et al*., 2002). However, JA has been reported to change the ratio between cytokinin ribosides and free bases and to

decrease the level of zeatin (Dermastia *et al*.,1994), which was proved to peak sharply before G2 / M transition (Redig *et al*., 1996). Moreover, exogenous supply of zeatin can rescue G2 arrest caused by the inhibition of cytokinin production (Laureys *et al*., 1998). But, Swiatek *et al*. (2002) stated that a block of G2 / M transition could not be recovered by zeatin treatment which means that the effect of JA is not directly linked with cytokinin action. The same authors revealed that both JA and ABA prevented DNA replication in tobacco cells, keeping the cells in the G1 stage, when applied just before the G1/S transition. But, when applied at a later stage, ABA had no effect whereby JA effectively prevented mitosis on application during DNA synthesis.

Novel results of Asahina *et al*. (2002), revealed that gibberellic acid is essential for cell division during the cortical repair of cut hypocotyls of cucumber and tomato. This result may refer to a possible interference of gibberellins in pathogenicity on the bases of some similarity between defense mechanisms against wounding and necrotrophic fungi (Hammond-Kosack & Jones, 2000). Some processes in wounding and pathogenic infection are recently known to be correlated via a small GTP-binding proteins that mediate cross signaling between the wound- and pathogen-induced signal transduction pathways (Sano *et al*., 1994; Sano & Ohashi, 1995; Zhou & Thornburg, 1999).

Work is also limited concerning relations between JA and auxin during defense processes in plants. However, auxin caused strong inhibition of methyl jasmonate-induced wound-inducible gene expression in soybean suspension cultured cells (DeWald *et al*., 1994) and expression of β-glucanase in response to fungal elicitor in tobacco and soybean (Jouanneau *et al*., 1991).

To our knowledge, only indirect evidence is available that cytokinins are essential for accumulation of wound-inducible proteinase inhibitor transcripts (Sano *et al*., 1994). Rather previously, wounding was proved to enhance endogenous cytokinin activity in cucumber (Crane & Ross, 1986).

Among the chemicals induced by pathogens are simple phenolic compounds that are mostly inhibitory to pathogens. Also, pathogens may produce glycosidases that can hydrolyze nontoxic phenolic glycosides to the toxic-free phenolic forms. In addition, polyphenol oxidases and peroxidases are activated by pathogens resulting in the oxidation of phenolics to form quinones, which are quite effective inhibitors of phytopathogens. In addition, a large number of toxic phytoalexins is derived from phenols (Orcutt & Nilsen, 2000).

Thus, this work intended to further study the effects of a low and a double fold concentration of JA on the defense potential of faba bean plants against infection with *Fusarium* wilt, with a particular emphasis on possible relationships with certain phytohormones. For this objective, ABA, GA_3, IAA, and the cytokinins zeatin and benzyladenine were studied. In addition, changes in phenolic compounds were followed.

MATERIALS AND METHODS

A pure cell line of faba bean (*Vicia faba* var. Giza 716) was obtained from the Agriculture Research Center, Ministry of Agriculture, Cairo, Egypt. Jasmonic acid (JA) was brought from Sigma Company and Rhizolex-T (Tolclofos- methyl- thiram) was brought from Sumitoma Chemical Company Ltd. Seeds were sterilized by 10% sodium hypochlorite. Treatments were done by soaking of seeds in JA solution at 5 mM or 10 mM, or the fungicide Rhizolex-T at 500 ppm for 8 h. A similar lot of seeds was soaked in water, representing controls. Then, the differently treated seeds were washed thoroughly with tap water, before sowing.

Pots (30 cm diameter) were filled with constant amounts of sterilized sandy loam soil (1:2 w/w). The prepared group (40 pots) was divided into two equal subgroups, each divided into four sets (control (H_2O), JA1(5 mM), JA2 (10 mM), and the fungicide-treated). Sowing was carried out in the first in sterilized soil, whereas in the second soil was infected with *Fusarium oxysporum* f. sp. *fabae* inoculum (5% of soil weight/pot). Irrigation of all pots was carried out routinely using a constant amount of tap water per pot. Pots were placed under natural conditions of daily light (11h) and darkness (13h), where the temperature varied from 20 ± 2°C during the day, 10 ± 2°C at night. The relative humidity varied from 60 to 80%. After 30 days, the different growth criteria were measured in the differently treated non-infected and infected plants. Results were statistically analysed to obtain the L.S.D (least significant difference) from the control (Snedecor & Cochran, 1967).

Mesurments of leaf area was directly detected by using Area Meter AM 100 (ADC Bioscieintific Limited, UK). Chlorophyll fluorescence was directly recorded, using the Chlorophyll Meter Minolta Spad- 502. The extraction, methylation and estimation of IAA, GA_3 and ABA were done according to the method sdopted by Guinn *et al.* (1986), and that of Müller *et al.* (1986). For cytokinins, in all cases, estimation was carried out using HPLC. Extraction and HPLC determination of simple phenols (caffeic, benzoic and P-coumaric acid) were done according to Malik and Singh (1980).

RESULTS AND DISCUSSION

Increases in jasmonic acid (JA) in response to pathogen attack occur both locally and systematically (Hammond-Kosack & Jones, 2000). These authors reported that a subset of inducible plant defense genes from *Arabidopsis* require a JA-dependent, salicylic (SA)-independent signaling pathway. Upon pathogenic fungal attack (especially in netrotrophic fungi), infected plant cells generate signaling molecules including JA to initiate

defense mechanisms surrounding cells to limit pathogen spread (Audenaert *et al*., 2002). It is recently well known that JA is subject to autoinduction, where exogenous application is associated with an increased level of endogenous JA (Maucher *et al*., 2000; Schaller, 2001). He *et al*. (2002), mentioned that JA biosynthesis is tightly regulated and the concentrations of JA in uninduced plant tissues are generally very low in most plant species examined.

In the present work, it was intended to find out if exogenous application of JA can account for different effects in non-infected faba bean plants (30-day-old) and those infected by Fusarium wilt (*Fusarium oxysporium f. sp. fabae*). In addition, the effect of a fungicide (Rhizolex-T) potent in controlling the fungus, was also investigated. The effects of JA and the fungicide on *Fusarium* wilt in faba bean plants will be discussed in the following:

Growth criteria. Table I shows that JA was slightly stimulating to the extension growth of shoot, particularly in the non-infected plants, as compared with corresponding control (H_2O). On the other hand, the fungicide seemed to be ineffective either in the infected or the non-infected plants. The root length (Table I) was slightly and obviously below corresponding control measures, in response to treatments with 5 mM and 10 mM JA, respectively. This result agrees with the widely known effect of JA to inhibit root growth in *Arabidopsis* (Swiatek *et al*., 2002). The authors mentioned that such effect was not directly linked with the wound and pathogen responses that are generally accepted to be the main function of jasmonates. There are also several reports suggesting a role of JA in cell wall synthesis in roots, which might negatively affect cell elongation (Koda, 1997). The fungicide did not significantly affect the root length in both non-infected and infected plants, as compared with controls. To further investigate possible effects of JA on the photosynthetic performance of faba bean plants, the number of leaves and total leaf area per plant, as well as chlorophyll fluorescence, were estimated. In non-infected plants, the above mentioned criteria were enhanced by the lower concentration of JA (5 mM), whereas double this concentration (10 mM) induced a lower effect. A reverse situation was shown in the infected plants where 10 mM JA showed a higher enhancement effect than the lower (5 mM). These results were evidently in alliance with the fresh and dry weight matter accumulation of these plants. In addition, the fungicide-treatment mostly induced a stimulatory effect on the number and total area of leaves per plant as well as the chlorophyll content, and consequently the fresh and dry weights of these plants. These results suggest that JA might achieve a dual effect on faba bean plants, depending on its applied concentration. This result is corroborated by the implementation of jasmonates as a class of plant growth regulators (Yoshihara, 1999). Recent molecular genetic studies have confirmed the involvement of JA both in developmental (Creelman & Mullet, 1995) and defense-related processes (Ryan, 2000). The present results clearly showed that the differential effects of 5 mM and 10 mM JA on the growth of faba bean plants was reversed in the non infected and infected plants. Thus, in the non-infected plants, 5 mM JA was markedly stimulatorier to growth, whereas in the infected plants, growth was evidently more favored by 10 mM JA. One plausible explanation is that the lower concentration of JA appeared likely to display a hormonal stimulatory effect in the uninfected plants. The higher concentration might induce an endogenous overproduced JA which would be sequestered, so that it could not exert a growth rate higher than that of the control (He *et al*., 2002). On the other side, in the infected plants, 10 mM JA was assumed to be adequate to fit the requirements for the defense pathway, but 5 mM might be below the threshold of triggering the signal cascade for the control of pathogenicity. This assumption could be reinforced by the insight that 10 mM JA could induce a full defense against the pathogen (more or less resembling the fungicide effect), whereby 5 mM JA was markedly less potent. The fungicide-enhancement effect on the above mentioned growth characters was similarly markedly more evident in the infected plants, as compared with uninfected plants.

Hormonal changes. It should be pointed that the features of hormonal changes are more intriguing in roots, which represent the primary site of action of *Fusarium* wilt attack.

Table I Effect of jasmonic acid at 5 mM (JA1) and 10 mM (JA2) and the fungicide Rhizolex-T on growth of faba bean plants (30-day-old), non infected and infected with *Fusarium* wilt. Each value is the mean of 10 replicates ± standard error

Infection	Conc. (mM)	Length of root (cm)	Length of shoot (cm)	No. of leaves / plant	Mean area of leaves / plant (cm^2)	Chlorophyll Fluoresc.	Fresh wt. (g / plant)	Dry wt. (g / plant)
Non - infected	O (H_2O)	11.33 ±1.46	21.20 ±2.79	5.8 ± 0.70	16.60 ± 2.95	41.82 ± 2.87	5.37 ±0.64	0.34 ± 0.029
	JA1	10.86 ±2.51	25.29 ± 0.72	6.6 ± 0.52	18.66 ±2.57	47.00 ±3.87	6.67 ±0.80	0.44 ±0.040
	JA2	11.86 ±1.27	24.14 ±1.01	6.2 ± 0.42	16.00 ±1.02	45.84 ±3.21	6.28 ±0.72	0.40 ±0.044
	Fungicide	10.60 ±1.78	21.34 ±1.58	6.0 ± 0.0	17.16 ±2.26	39.54 ±3.50	5.43 ±0.87	0.36 ±0.043
LSD.05		N.S.	1.681	4.28	N.S.	3.84	0.68	0.11
Infected	O (H_2O)	10.59 ±1.67	20.40±1.08	5.8 ± 0.41	12.89 ±1.79	36.26 ±4.51	4.79 ±0.42	0.32 ±0.03
	JA1	10.70 ±1.03	22.91±1.93	6.7± 0.48	19.30 ±2.32	43.44 ±0.79	6.04 ±0.54	0.42 ±0.045
	JA2	9.19 ±1.06	24.10 ±1.26	6.8± 0.42	22.40 ±2.97	44.60 ±0.64	6.39 ±0.32	0.49 ±0.011
	Fungicide	10.48 ±3.25	20.90 ±0.67	6.3 ± 0.42	18.19 ±1.72	39.40 ±0.85	5.95 ±1.0	0.45 ±0.061
LSD.05		N.S.	1.88	0.64	3.08	3.5	0.60	0.05

Table II. Levels of abscisic acid (ABA), gibberellic acid (GA_3), indoleacetic acid (IAA), and GA_3/ABA ratios in roots and shoots of faba bean plants (30-day-old), non-infected and infected with *Fusarium* wilt. In both cases, treatments were carried out using 5 mM (JA1) and 10 mM (JA2) jasmonic acid and the fungicide Rhizolex-T. Results are expressed as mg/100 g dry wt. equivalents

Treatments	Non – infected				Infected			
	ABA	GA_3	GA_3/ABA	IAA	ABA	GA_3	GA_3/ABA	IAA
A. Roots								
Control (H_2O)	116.2	648.3	5.58	6.30	369.5	177.5	0.48	6.2
JA1	133.6	2602.0	19.47	0.97	382.5	1115.3	2.91	10.8
JA2	233.8	212.8	0.91	3.75	53.8	2053.8	38.20	5.2
Fungicide	505.5	2431.1	4.81	8.03	192.3	486.3	2.53	4.3
B. Shoots								
Control (H_2O)	945.1	1346.8	1.42	14.92	1136.9	1412.0	1.24	7.9
JA1	104.1	2844.7	27.35	52.83	150.4	2281.8	15.17	50.0
JA2	702.1	3459.9	4.93	41.72	944.6	2175.6	2.30	7.9
Fungicide	879.5	1359.3	1.55	14.19	1206.6	2135.3	1.77	35.6

The data in Table II show that in the non-infected plants, JA treatments resulted in a higher ABA levels in roots than that corresponding control plants. This content was directly proportional to the applied concentration of JA. On the other hand, ABA content in shoots of the JA-treated plants was markedly below than that of the control. However, the substantially lower content of ABA in shoots of the plants treated with 5 mM JA than in those treated with 10 mM was in alliance with the higher growth rate of the formers than the laters. In this connection, ABA is known to participate in several processes including the regulation of plant growth and development, particularly via impact on the major elements of the dynamic cytoskeleton. In this connection, ABA tends to be antagonistic to both auxins and gibberellins with respect to their effect on the arrangement of cytoskeleton microtubules (CMTs) and cell growth (Baluska *et al*., 1999). These authors mentioned that the most characteristic effect of ABA on CMTs is to induce longitudinal MT orientations, which is commonly associated with the cessation of cell growth. ABA was found to up-regulate a protein named ICK1 (inhibitor of cyclin-dependent kinase), which leads to a block of G1/S transition (Wang *et al*., 2000). There is also some evidence that ABA is included with programmed cell death and hypersensitive responses within the pathogen-infected sites (Dangl *et al*., 2000).

Table II also shows that in response to application of the fungicide in absence of the fungus infection, ABA level was several folds higher in roots but lower in shoots, as compared with those of the control (non-infected) plants. However, in both cases (shoots and roots), ABA was markedly higher on application of the fungicide than both JA treatments. In this respect, the fungicide might represent a stress signal in the uninfected plants. In this respect, ABA is proved to be included in the protection against bursts of superoxide and /or hydrogen peroxide as reactive oxygen species (ROS) under stress- induced oxidative damage in *Arabidopsis* (Larkindale & Knight, 2002). The production of ROS is often the first response detected, occurring within few minutes during pathogen attack (Hammond-Kosack & Jones, 2000).

In the infected plants, the ABA content of roots was slightly higher than in the control plants, in response to treatment with 5 mM JA; whereas, a dramatic drop was shown in treatment with 10 mM JA. The results obtained with 10 mM JA (which achieved complete defense against *Fusarium* wilt) might suggest that the defense response against *Fusarium* wilt is concomitant with a depression in ABA level in roots. This concept might be accepted on the basis that the root is the local injury site, and consequences in terms of gene action responsive to elicitor signals and allied transduction pathways. Secondly, increased ABA levels were observed in response to infection with viruses, bacteria, and fungi (Kettner & Dorffling, 1995). It is also generally found that application of exogenous ABA increases the susceptibility of plants to fungal pathogens (McDonald & Cahill, 1999). In this respect, ABA was found to suppress phenylalanine ammonia lyase (PAL) activity and transcription of PAL mRNA in hypocotyls inoculated with the incompatible pathogen *Phytophthora megasperma* f. sp. *glycinea* (Ward *et al*., 1989). Moreover, physiological ABA concentrations down- regulated PAL at the level of transcription in tobacco (*Nicotiana tabacum*) cell cultures, where this enzyme is implicated in responses to wounding and pathogen infection (Rezzonico *et al*., 1998).

In the infected plants, the fungicide treatment caused a marked decrease in ABA content of roots and a slight increase in shoots, as compared with corresponding control values (Table II). Thus, it was apparent that the changes in ABA levels in the infected plants had similar trends in roots with both the fungicide-treated and 10 mM JA-treated plants. However, in the non- infected plants, a similar conclusion was also obtained.

To our knowledge, an ABA-dependent defense-signaling pathway has not been documented yet. Thus, the results obtained in the present work might conclude that ABA is assumed to have a cross talk with JA-induced defense in faba bean plants against *Fusarium* wilt. It is suggested that if the defense mechanism is potential and

Table III. Cytokinin contents, as zeatin and benzyladenine and their total values, in roots and shoots of faba bean plants (30-day-old), non-infected and infected with *Fusarium* wilt. In both cases, treatments were carried out using 5 mM (JA1) and 10 mM (JA2) jasmonic acid and the fungicide Rizolex-T. Results are expressed as mg/100 g dry wt. equivalents

Treatments	Non – infected			Infected		
	Zeatin	Benzyl adenine	Total	Zeatin	Benzyl Adenine	Total
A. Roots						
Control (H_2O)	1.56	246.17	247.73	1.84	377.46	379.30
JA1	1.68	743.44	745.12	0.66	2268.04	2268.70
JA2	2.68	2647.02	2649.70	1.04	4657.50	4658.54
Fungicide	0.34	456.37	456.71	2.61	105.00	107.61
B. Shoots						
Control (H_2O)	13.40	2227.50	2240.90	0.59	261.74	262.33
JA1	5.44	760.16	765.60	14.92	1240.36	1255.28
JA2	5.19	1211.42	1216.61	12.56	1328.82	1341.38
Fungicide	11.31	197.43	208.74	6.63	934.39	941.02

Table IV. Phenolic contents, as caffeic, benzoic and p-coumaric acids and their total values, in roots and shoots of faba bean plants (30-day-old), non-infected and infected with *Fusarium* wilt. In both cases, treatments were carried out with 5 mM (JA1) and 10 mM (JA2) jasmonic acid and the fungicide Rhizolex-T. Results are expressed as mg/100 g dry wt. equivalents

Treatments	Non - infected				Infected			
	Caffeic acid	Benzoic acid	p-Coumaric acid	Total	Caffeic acid	Benzoic acid	p-Coumaric acid	Total
A. Roots								
Control (H_2O)	95.50	49.11	5.10	149.71	11.55	7.82	1.09	20.46
JA1	28.42	29.92	0.86	59.20	23.33	174.92	4.36	202.61
JA2	33.76	10.60	8.43	52.79	94.58	29.09	7.17	130.84
Fungicide	93.46	12.69	1.61	107.76	16.96	31.16	2.61	50.73
B. Shoots								
Control (H_2O)	66.10	71.80	11.33	149.23	25.58	72.18	22.33	120.09
JA1	94.20	197.46	13.11	304.77	89.26	167.02	2.92	259.2
JA2	66.94	160.88	8.36	236.18	86.91	115.50	12.97	215.38
Fungicide	66.50	94.23	5.48	166.21	82.47	90.92	9.62	183.01

adequate to overcome the pathogen, a depression in ABA will take place in roots. But, if the conditions are favoring infection, ABA will cross a threshold level. However, these suggestions give a fragmentary picture and provide few or indirect clues for the mechanistic basis of the involvement of ABA with JA in plant defense.

The results presented in Table II generally outlines a reverse relation between the contents of ABA and those of GA_3. However, the GA_3/ABA ratio could clarify some features of certain significance. In the control plants, this ratio was substantially decreased in response to the pathogen infection in roots, with clear differences in shoots. In the non-infected plants, the GA_3/ABA ratio was evidently enhanced in treatment with 5 mM JA, particularly in shoots, which is in alliance with the stimulation of the growth rate of these plants. But, in treatment with 10 mM JA, the ratio observed was substantially lower than that of the control in roots but obviously higher in shoots. On the other hand, in the infected plants the GA_3/ABA ratio showed several folds increase in roots as a result of the potential defense induced by 10 mM JA, whereby a slight increase was observed in shoots. A reverse situation was shown in treatment with 5 mM JA where this ratio was markedly higher in shoots and dramatically lower in roots, as compared with those of corresponding controls. Table II also shows that the fungicide treatment did not cause a marked changes of the GA_3/ABA ratios, as compared with the controls, in both roots and shoots. But, in the infected plants this value was obviously increased in both organs. On these basis, it appeared likely that the overcoming of *Fusarium* pathogenicity in faba bean roots, as a result of treatment with 10 mM JA, was underlined with a sharp increase of the GA_3/ABA ratio. This might be, however, supported to a certain extent by the novel results of Asahina *et al*. (2002), which revealed that GA is essential for cell division during the cortical repair of cut hypocotyls of cucumber and tomato. This interpretation is given, herein, on the basis of general information concerning some similarity between defense mechanisms against wounding and necrotrophic fungi (Hammond-Kosack & Jones, 2000).

The changes in the auxin IAA (Table II) show that in the non- infected plants, JA treatments were concomitant with a decrease in roots, particularly with the lower concentration (5 mM JA) and an enhancement in shoots, especially that lower concentration. On use of the fungicide, IAA content was slightly increased in roots and comparable to the control level in shoots. In the infected plants, on the other side, the defense shown in treatment with either 10

mM JA or the fungicide was peculiar to a decrease of IAA content in roots; whereas, an increase was recorded in treatment with 5 mM JA. In shoots, inconsistent results were shown. This might indicate a reverse relation between potential defense and the IAA content in roots of the pathogen-infected plants. This conclusion could be reinforced by the results which revealed strong inhibition by auxin of methyl jasmonate-induced wound-inducible gene expression in soybean suspension cultured cells (DeWald *et al*., 1994) and expression of β-glucanase in response to fungal elicitor in tobacco and soybean (Jouanneau *et al*., 1991). The cellular machinery during wounding and pathogenic infection is recently known to be correlated via a small GTP-binding proteins that mediate cross signalling between the wound- and pathogen-induced signal transduction pathways (Sano *et al*., 1994; Sano & Ohashi, 1995; Zhou & Thornburg, 1999).

The results in Table III show that JA induced a marked enhancement of the total cytokinin content in roots of either the infected or non-infected plants. In both cases, as well as in shoots, the magnitude of increase was directly proportional with the applied concentration of JA. On treatment with the fungicide, an increase was also recorded in roots of the non-infected plants, whereby a decrease was observed in roots of the infected plants. However, this decrease was concomitant with prominently evoked levels in shoots. However, the reversed levels of total cytokinins in roots and shoots seemed likely to indicate an effect of the different applied treatments on cytokinin partitioning among the two organs. The above-mentioned patterns in total cytokinin contents were obviously in alliance with those in benzyladenine that constituted the major cytokinin fraction. However, zeatin content was obviously increased by both JA treatments, except in roots of the infected plants. In this connection, JA has been reported to decrease the level of zeatin (Dermastia *et al*., 1994). Moreover, exogenous supply of zeatin can rescue G2 arrest caused by the inhibition of cytokinin production (Laureys *et al*., 1998). This may further reinforce our conclusion that the effects of JA differ in normal plants and those subjected to infection. To our knowledge, work in this respect is limited, but there is indirect evidence that cytokinins are generally essential for accumulation of wound-inducible proteinase inhibitor transcripts (Sano *et al*., 1994). Rather previously, wounding was proved to enhance endogenous cytokinin activity in cucumber (Crane & Ross, 1986).

Phenolic compounds . Table IV shows the changes in some phenolic compounds in response to JA and fungicide treatments in non-infected and infected faba bean plants. The results obtained indicate that in roots of the non-infected plants, phenolic compounds were markedly decreased as a result of JA and fungicide treatments; whereas, a reverse situation was recorded in shoots. In the infected plants, on the other hand, the total value of the detected phenols, in roots, showed highest level in response to 10 mM JA followed by 5 mM JA then by the fungicide treatment. A similar trend was also observed in shoots. The above mentioned changes in phenolic compounds could be attributed to changes in the three detected phenolic substances, caffeic, benzoic, and coumaric acids. This result agrees with the general speculation that when cells are recruited into infection, switch from normal primary metabolism to a multitude of secondary metabolism defense pathway and activation of novel defense enzymes and genes takes place (Hammond-Kosack & Jones, 2000). General up-regulation of genes encoding the phenylpropanoid pathway is recorded in order to provide the cell with lignin precursors and phenolic plant-defensive compounds (Zhou & Thornburg, 1999). It should be mentioned that defense signals resulting in increased phenolic and other phytoalexin compounds could be induced by jasmonates (Baldwin, 1996).

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