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EFFECT OF SALICYLIC ACID SPRAYING ON PHYTOALEXINS SYNTHESIS OF SUGAR PEAS AND ITS RELATION TO POWDERY MILDEW DISEASE INCIDENCE AND YIELD.

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ABSTRACT

Accumulation of phytoalexins is a host defense mechanism against invading pathogens. It is not clear if phytoalexin can be produced in sufficient quantity by exogenous treatment of plants with elicitors. Two experiments were conducted during winter season of 2004/05 and 2005/06 in Nubaria area, Alex-Desert Road to investigate the effect of salicylic acid (SA) as an inducer/elicitor of pisatin in pea and chemical compounds known to be involved in conferring resistance against pea powdery mildew as well as yield improvement. Salicylic acid (SA) was applied at 0.02, or 0.04 or 0.06% concentrations to six-week-old sugar peas plants to determine if it could induce the synthesis of pisatin, (the pea phytoalexin). There was an increase in the phenylalanine ammonia-lyase activity and phenolic contents in plants treated with SA. Plants treated with 0.02 and 0.04% SA showed minor variations in phenylalanine ammonia-lyase activity and phenolic contents and did not accumulate detectable amounts of pisatin. Treatment with 0.06% SA increased pisatin and phenolic contents in susceptible and resistant pea plants. Reduction in powdery mildew disease incidence as well as increasing in pod yield were recorded. TSS content didn't affect by the treatments in this study.

INTRODUCTION

Chemical control mostly causes environmental pollution, highly affects the growth of the host plants, lead to great disturbance of the natural biological balance and the most serious result in the great increase in the accumulation of the toxic substances in the human food chain.

Plants have developed an array of defense strategies to respond to microbial attack. The systemic acquired resistance is an important component of plant's defense against diseases, where initial infection provides systemic resistance to subsequent infection by a variety of bacterial, fungal and viral pathogens (Gaffiney *et al.,* 1993). These include the rapid production of reactive oxygen species intimately associated with the hypersensitive response, the accumulation of antimicrobial phytoalexins and pathogenesis related proteins, and the strengthening of the cell walls by deposition of lignin and hydroxypriline-rich proteins (Dixon *et al.,* 1994).

Over the past twenty years, induced disease resistance was demonstrated in a number of plant-pathogen systems by using biotic and abiotic inducing agents (Dean and Kuc, 1985; Doubrava *et al.*, 1988; Chen *et al.*, 1993; Dekker, 1996; Chondra *et al.*, 2001 and Fouly, 2004). The major hallmark of this form of resistance is the ability of the plants to defend themselves against a broad spectrum of pathogens by triggering plant species-specific defense responses (Metraux, 2001). The signal molecules that begin the process of phytoalexin synthesis in plants are called 'elicitors.' Both biotic (complex carbohydrates from fungal and plant cell walls) and abiotic (heavy metals, salts and UV light elicitors have been reported to induce phytoalexin synthesis (Sticher *et al.*, 1997). The isoflavonoid phytoalexin 'pisatin' is formed in pea (*Pisum sativum* L.,) in response to biotic and abiotic stimuli. The first report on the structure of pisatin from pea was reported in the early 1960s (Hammerschmdidt, 1999).

The use of salicylic acid to induce systemic acquired resistance was previously used to minimize the infection with many diseases (Malamy and Kessing, 1992; Dekker, 1996; Sticker *et al.*, 1997; Ibrahim, 1998; Bhatt *et al.*, 1999; Abou-Taleb, 2001; Pradeep and Jambhale, 2002; Attia, 2004 and Fouly, 2004)

The infection of pea with powdery mildew, caused by the fungus *Erysiphe pisi* (Green, 1991) is a devastating disease of pea which reduces yield and quality of the pods (Munjal *et al.*, 1963, and Salama, 1990), thus, incurring huge economic losses to the growers.

Sugar peas became occupying a significant area in Egypt for fresh consumption. The area reached 2318 feddan in winter season of 2005 (Min.of Agric., 2005, annual report).

The objective of the present study was to investigate the effect of salicylic acid (SA) as inducer/elicitor of pisatin in pea and chemical compounds known to be involved in conferring resistance against plant pathogens as well as yield improvement. This is the current strategy of controlling pests of vegetables and fruits which depends on using alternative methods of disease control rather than pesticides and fungicides especially for fresh consumption products.

MATERIALS AND METHODS

Two field experiments were conducted during the winter season of 2004/05 and 2005/06 in Nubaria area, Alex-Desert Road. Soil was sandy in texture with pH of 8.1. The objective of these experiments was to study effect of salicylic acid spraying as an inducer/elicitor of pisatin in pea plants and chemical compounds known to be involved in conferring resistance against powdery mildew disease as well as yield improvement.

It is worth to mention that field observations revealed that Matabea and Mozellae pea cvs. are susceptible and resistant, respectively to pea powdery mildew caused by *Erysiphe pisi*.

Under natural infection, adjacent sugar peas field with a history of powdery mildew, seeds of the sugar peas (*Pissum sativum* var.*macrocarpon*) cvs. Matabea and Mozellae, (Tezier Co., France), previously inoculated with *Rhizobium leguminosarum*, were sown on 15th and 17th of October for the first and second seasons, respectively.

The employed treatments in this study were:

Salicylic acid treatment (SA):

Six-week-old plants were treated with 0.02, or 0.04 or 0.06 % solutions of SA(dissolved in acetone just before spraying) applied by spraying unit runoff. Control plants were treated with distilled water (dissolved in acetone just before spraying) . The top five leaves of the plants were sampled at 0, 24, 48, 72, 96 and 120 hr after treatment and analyzed for phenylalanine ammonia-lyase (PAL) activity and pisatin and phenolic content. Each treatment and sampling time combination had three replications.

A split-plot design with three replicates was used in this study. The main plots were assigned for two cultivars and the sub-plots were occupied by the tested SA applications. Unsprayed plants with SA served as a control. So, eight treatments were involved in this study. Each sub-plot was 18m^2 included 2 beds, 1.5m wide and 6m long. Plants were spaced 15cm on the two sides of the bed (as recommended by the producer, Tezier Co. for these sugar peas cultivars).

At the time of soil preparation, 10m^3/fed of farm-yard manure were added 30cm deep along planting beds, mixed with 300, 100 and 50kg/fed of calcium superphosphate, potassium sulfate and ammonium nitrate, respectively. During growing season, all experimental units were fertilized with 60 liters of phosphoric (60 % P_2O_5), 50 kg of potassium sulfate (48 % K_2) and 50 kg/fed of ammonium nitrate(33.5 % N) per feddan. They were likewise applied in weekly doses through the drip irrigation system used in this study, beginning 2 weeks after germination (4 true leaf stage).

Data were recorded on the following parameters:

1. Biochemical analysis:

Ten plants for each experimental unit were chosen randomly and the top five leaves of the plants were sampled at 0, 24, 48, 72, 96 and 120 hr after treatment and analyzed for phenylalanine ammonia-lyase (PAL) activity and pisatin and phenolic content. Each treatment and sampling time combination had three replications.

1.1. Pisatin analysis:

Pisatin was extracted from the samples using the method developed by Oku *et al.* (1975). A UV spectrophotometer was used to scan the absorbance of the samples between 280 and 320nm. For the samples exhibiting the characteristic absorbance scan of pisatin, with a major peak at 309nm, the A_{309nm} value was used to calculate the concentration in mg/g fresh wt. from the formula:

$$C = A \times MW/\varepsilon$$ (Oku *et al.*, (1975)

Where : 'C' is the concentration,
'A' is the absorbance at 309nm in ethanol,
MW is the molecular weight of pisatin (314 g/mole), and
ε is the molar extinction coefficient of pisatin in ethanol
(ε = antilog 3.86 = 7244 L/mole).

1.2. Phenylalanine ammonia-lyase activity (PAL):

Pea leaf samples (250 mg) were homogenized in a pre-chilled pestle and mortar with glass capillaries as the grinding material and 5 mL ice cold of 0.1 M HCI buffer (pH 7.5). Extracts were centrifuged for 25 min. and the supernatant used as the enzyme extract for determination of PAL activity according to the method by Burrell and Rees (1974).

1.3. Estimation of phenolic compounds:

Dried pea leaf samples (40 mg) were refluxed with 5 mL of hot 80% methanol for one hour. The refluxed material was filtered and the volume brought to 10 mL by washing with hot 80% methanol. The extract was used for estimation of total phenols (Swain and Hillis, 1959)

2. Disease incidence (%):

Plants /each experimental unit were examined weekly for disease symptoms development. Percentage of diseased and infected plants of the total plants/experimental unit (as percent.) was assessed using the devised scale by Horsfall and Barratt (1945), then the averages were calculated.

3. Average yield and TSS content:

The produced pods of each experimental unit were harvested periodically (when it was necessary and at apparent market maturity, 80 days after planting), weighed and the averages were recorded (kg/m^2). The harvest period continued 2 months. For TSS estimation, ten pods were taken randomly/each treatment /each harvesting to determine their total soluble solids (TSS%) using a hand refractometer and the averages were recorded.

Statistical analysis:

Data were statistically analyzed using the split plot design (Snedecor and Cochran, 1967). The averages were compared at 5% level of probability by the L.S.D. test.

RESULTS AND DISCUSSION

1. Biochemical analysis:

1.1. Pisatin content:

Pisatin content in the pea leaves after treatment with SA is shown in Tables (1 and 2). Treatment of six-week old control plants, and those treated with 0.02 and 0.0.4 % SA, indicated no detectable amount of pisatin formed up to 120 hr after treatment, but when plants were treated with 0.06 % SA, 14.5 and 18.8 µg/g fresh wt pisatin was detected 48 and 72h after treatment, respectively and its content increased up to 32.5 µ/g fresh wt 120 hr (2004/05 season). The same trend was true in the next season.

Table (1): Pisatin content in leaves of sugar peas after treatment with salicylic acid, 2004/05 season.

Treatments	Pisatin content (mg/g/fresh wt.) Hours after treatment					
	0	24	48	72	96	120
Cultivars :						
Matabea	0.7	0.7	3.1	4.3	5.1	7.5
Mozellae	0.7	0.7	6.5	6.2	7.5	9.7
L.S.D. at (0.05):	n.s.	n.s.	1.3	1.4	1.4	1.4
SA application:						
Control	0.4	0.4	0.5	0.7	0.6	0.4
0.02% SA	0.6	0.7	0.6	0.8	0.7	0.8
0.04% SA	0.9	0.9	0.7	0.9	0.8	0.9
0.06% SA	0.9	0.9	14.5	18.8	24.1	32.5
L.S.D. at (0.05):	n.s.	n.s.	2.7	2.8	2.8	2.5
Interaction						
Matabea:						
Control	0.4	0.3	0.5	0.6	0.4	0.2
0.02% SA	0.6	0.7	0.6	0.7	0.6	0.9
0.04% SA	0.9	0.9	0.7	0.8	0.8	0.9
0.06% SA	0.9	0.9	10.8	15.3	20.4	28.3
Mozellae:						
Control	0.4	0.4	0.5	0.8	0.8	0.6
0.02% SA	0.6	0.8	0.6	0.9	0.8	0.7
0.04% SA	0.8	0.9	0.8	0.9	0.8	0.9
0.06% SA	0.9	0.9	18.3	22.4	27.8	36.8
L.S.D. at (0.05):	n.s.	n.s.	2.8	2.9	2.9	2.8

Regarding to cultivars content of pisatin, data in Tables, (1 and 2) show that pisatin synthesis started quickly and at about the same time (48 hr) in both susceptible(3.1 µg/g) and resistant(6.50µg/g) cultivars and there was a gradual increase in the content up to 120 hr (2004/05 season).

Similarly, with some extent, the same trend was observed due to the interaction between cultivars and SA treatments, pisatin accumulation started quickly and at about the same time (48 hr) in both susceptible(10.8 µg/g) and resistant(18.3 µg/g) cultivars and there was a gradual increase in the content up to 120 hr (2004/05 season). However, treatment with 0.06% SA resulted in higher content of pisatin in the resistant cultivar (Mozellae) compared with the susceptible one i.e., Matabea (Tables, 1 and 2).

Similar results were obtained by Smith *et al.*(1975) who reported that these difference could be due greater efficiency of phytoalexin synthesis in the resistant plants. Also, Rusch and Laurence (1993), reported that the faster accumulation of defense molecules by the plants may be one of the major factors used to defend against infection.

Table (2): Pisatin content in leaves of sugar peas after treatment with salicylic acid, 2005/06 season.

Treatments	Pisatin content (mg/g/fresh wt.) Hours after treatment					
	0	24	48	72	96	120
Cultivars :						
Matabea	0.6	0.6	3.2	4.2	5.5	7.8
Mozellae	0.6	0.7	5.1	5.8	7.2	9.6
L.S.D. at (0.05):	n.s.	n.s.	1.2	1.3	1.3	1.4
SA application						
Control	0.3	0.3	0.4	0.5	0.3	0.3
0.02% SA	0.5	0.6	0.6	0.7	0.7	0.7
0.04% SA	0.7	0.8	0.6	0.9	0.9	1.2
0.06% SA	0.9	0.9	14.8	17.9	23.6	32.6
L.S.D. at (0.05):	n.s.	n.s.	1.5	1.7	1.5	1.8
Interaction						
Matabea:						
Control	0.3	0.3	0.5	0.4	0.2	0.2
0.02% SA	0.5	0.6	0.6	0.8	0.8	0.9
0.04% SA	0.7	0.7	0.8	0.9	0.9	1.1
0.06% SA	0.9	0.9	11.1	14.9	20.2	29.1
Mozellae:						
Control	0.3	0.3	0.4	0.5	0.5	0.5
0.02% SA	0.6	0.7	0.5	0.7	0.6	0.5
0.04% SA	0.8	0.8	0.5	0.9	0.9	1.3
0.06% SA	0.9	0.9	18.6	21.0	27.1	36.2
L.S.D. at (0.05):	n.s.	n.s.	1.7	1.9	1.9	1.8

As Darvill and Albersheim (1984), mentioned that plants employ various biosynthetic pathways to synthesize these secondary metabolites including phytoalexins. A single plant may employ as many as 20 enzymes to synthesize phytoalexins. These enzymes are either absent, or present in very low levels, in healthy, unelicited tissues and their level rise soon after elicitation. Accumulation of phytoalexins is one of the disease resistance mechanisms in plants. A variety of substances can induce the synthesis of secondary metabolites to a variable extent. Orober *et al.* (2002) indicated high level of systemic protection in cucumber plants against anthracnose after treatment with dipotassium hydrogenphosphate (K_2HPO_4).

1.2. Phenylalanine ammonia-lyase activity (PAL):

By 24 hr PAL activity increased gradually after treatment with 0.02 and 0.04% SA, but after treatment with 0.06% SA, PAL activity values were higher as compared to the control plants (Tables, 3 and 4). In this respect, Darvill and Albersheim (1984) reported that PAL is the key enzyme in the biosynthetic pathway of isoflavonoid phytoalexin production in pea. Therefore, an increase in its activity is related to increase in pisatin content. The inhibition of enzyme leads to the decrease in the phytoalexin content as well as to the loss of resistance to the pathogens (Darvill and Albersheim, 1984). The correlation of phytoalexin synthesis with PAL activity showed that inhibition of PAL by L-2-amino3-phenylpropanoic acid leads to loss of resistance to *Phytophthora megasperma*, the fungal pathogen that causes root and stem rot in soybean (Darvill and Albersheim, 1984). Similarly, inhibition of the enzyme leads to a decrease in phytoalexin content as well as to the loss of resistance to the pathogen (Moesta and Grisebach, 1982). In the present study, 0.06% concentration of SA induced the synthesis of pisatin after 72 hr of treatment with a concurrent increase in PAL activity started after 24 hr. Pisatin accumulation was detected after 72 hr indicating that the phytoalexin synthesis does not occur for some time after treatment with the elicitor. This may be due to the induction of the different enzyme involved in the synthesis of pisatin. It has been shown in peas that accumulation of phytoalexin upon challenge by an elicitor results from the de novo synthesis of the enzyme that catalyzes the synthesis of phytoalexins (Loschke *et al.*, 1981). Furthermore, evidence has been obtained in parsley cells that, as a result of elicitation of phytoalexins the mM RNA that encode the enzyme required for the synthesis of phytoalexin are themselves synthesized *de novo* (Ragg *et al.*, 1981). Elicitors appear to stimulate phytoalexin synthesis by activating expression of genes responsible for encoding the enzymes required for the synthesis of phytoalexins (Dean and Kuc, 1985). Also, Kessmann *et al.* (1994) mentioned that the accumulation of phytoalexins at the site of attempted infection is one of the mechanisms by which plants resists disease. PAL activity in 'Mozellae' treated with 0.06% SA was higher after 24 hr than in control plants or other SA concentrations and the same trend was recorded in Matabea cv. (Tables, 3 and 4). Similar results were obtained by Chen *et al.*(1993) and Chondra *et al.*(2001), they reported that PAL is the major enzyme in the synthesis of pisatin.

1.3. Total phenols content

Total phenols showed variable changes in their contents after treatment with different SA concentrations, as compared to the control leaves. In most of the cases their contents were higher in treated than the untreated leaves (Tables, 5 and 6). In all cases by 72 hr all treated plants had higher levels of total phenols than control plants. Phenolic compounds and their precursors are toxic for the invading pathogens; therefore, their higher content in the plants is one of the important components for conferring resistance against the disease (Sindhan and Parashar, 1996). Phenolic are involved in responses to UV radiations, environmental pollutants, nutritional deficiency, pathogens and herbivores in the absence of or in support of other kinds of defense mechanisms that may be preformed subsequently (Beckman, 2000).

The phenolic contents were higher in the resistant cultivar (Mozellae) as compared to the susceptible cultivar (Matabea), and the increase was more with 0.06% SA as compared to 0.02 and 0.04% SA concentrations. Karthikeyan and Narayanaswamy (1989) also observed higher levels of phenols in rice resistant cvs. to *Rhizoctonia solani*. Furthermore, Abo-Taleb (2001) reported that among the secondary plant products phenolic compounds are an important group implicated in both constitutive and induced resistance. Presence of phenols and their oxidation products in the plants are toxic to the growth and development of pathogens. Therefore, an increase in the phenolic contents in the resistant cultivar after inducer treatments contributes for the resistance against disease (Pradeep and Jambhale 2002).

Table (3): Phenylalanine ammonia-lyase activity (PAL) in leaves of sugar peas after treatment with salicylic acid, 2004/05 season.

Treatments	PAL activity (mg/g/fresh wt.) Hours after treatment					
	0	24	48	72	96	120
Cultivars :						
Matabea	129	150	197	220	218	243
Mozellae	235	242	253	265	280	301
L.S.D. at (0.05):	15	17	19	18	22	26
SA application:						
Control	177	181	196	212	198	199
0.02% SA	180	192	219	233	242	248
0.04% SA	184	199	227	240	248	254
0.06% SA	187	213	257	285	308	386
L.S.D. at (0.05):	n.s.	14	16	19	22	28
Interaction:						
Matabea:						
Control	125	140	180	201	172	181
0.02% SA	127	143	186	203	210	217
0.04% SA	130	153	198	210	215	223
0.06% SA	135	165	225	267	275	351
Mozellae:						
Control	230	222	212	223	225	218
0.02% SA	234	241	252	263	275	279
0.04% SA	238	245	257	270	281	286
0.06% SA	239	261	289	303	341	422
L.S.D. at (0.05):	15	16	22	23	27	32

2. Disease incidence (%):

Data presented in Table (7) show that SA treatments generally resulted in a significant reduction in powdery mildew disease incidence with significant increase the pod yield compared with the control. Moreover, 0.06% SA was more efficient than other SA concentrations. No significant differences observed between SA concentrations of 0.02 and 0.04%. The control treatments recorded highest disease incidence. Regarding to the interaction treatments, data in the same Table (7) revealed that the average of disease incidence on unsprayed plants with SA in "Matabea" cv. was 10.1% in 2005/6 season but 0.06 % SA treatment reduced disease incidence to be 3.5%. The same trend was observed in Mozellae cv. Significant differences in disease incidence were observed between cultivars. It is logical result because Mozellae cv. is known as a resistance cv. and Matabea is a susceptible cv.

The reduction in powdery mildew disease incidence herein supports the hypothesis obtained by Fouly (2004), who reported that induced acquired resistance induced by restricted infection is not due to a specific component of the pathogen, but rather to gradual appearance and gradual persistence of a level of metabolic perturbation leading to stress on the host.

Table (4): Phenylalanine ammonia-lyase activity (PAL) in leaves of sugar peas after treatment with salicylic acid, 2005/06 season

Treatments	PAL activity (mg/g/fresh wt.) Hours after treatment					
	0	24	48	72	96	120
Cultivars :						
Matabea	136	151	202	225	223	242
Mozellae	235	243	249	257	277	297
L.S.D. at (0.05):	15	17	17	17	18	19
SA and inoculation application						
Control	177	173	190	206	200	203
0.02% SA	183	193	220	230	231	238
0.04% SA	188	200	230	249	257	266
0.06% SA	195	222	262	280	314	373
L.S.D. at (0.05):	14	16	15	15	18	18
Interaction						
Matabea:						
Control	128	131	181	203	186	187
0.02% SA	137	148	192	209	193	205
0.04% SA	139	153	197	219	225	231
0.06% SA	142	172	239	272	291	348
Mozellae:						
Control	226	215	199	210	215	219
0.02% SA	230	239	248	252	269	271
0.04% SA	237	247	263	279	289	301
0.06% SA	249	273	286	289	338	399
L.S.D. at (0.05):	14	16	15	16	19	18

Also, Dean and Kuc (1985) indicated that induced acquired resistance is persistent and gradually is pathogen nonspecific. In addition, systemic acquired resistance can be induced by simple substances as well as by biotic agents (Doubrava *et al.* (1988). On the other hand, Lancke (1981) found that unlike elicitors of phytoalexins accumulation, which are elicited at the site of application may be responsible for localized protection and induces systemic acquired resistance that sensitizes the plant response rapidly after infection. These response induced phytoalexins accumulation and lignifications (Dean and Kuc, 1985 and Kuc and Rush, 1985) and induce or enhance activities of Chitinase and B-glucanase (Metreux and Boller, 1986). Furthermore, Kessmann *et al.* (1994) mentioned that the mechanism of systemic acquired resistance is apparently multifaceted, likely resulting in stable, broad spectrum disease control and they could be used preventatively to bolster general plant health, resulting in long lasting protection.

Chen *et al.* (1993) and Chondra *et al.* (2001) cloned and sequenced the salicylic acid binding protein which exhibited catalase activity. They also reported that the action of salicylic acid in systemic acquired resistance is obtained by elevated amounts of H_2O_2 by inhibiting the catalase activity. Apart from this, it has also been reported that salicylic acid induces many proteins similar in nature to the pathogenesis related proteins (Linthorst, 1991).

Table (5): Total phenols in leaves of sugar peas after treatment with salicylic acid, 2004/05 season.

Treatments	Total phenols (mg/g/dry wt.) Hours after treatment					
	0	24	48	72	96	120
Cultivars :						
Matabea	8.2	9.7	9.8	12.3	14.5	16.4
Mozellae	12.5	12.7	12.8	16.6	18.7	22.1
L.S.D. at (0.05):	1.5	1.5	1.6	1.5	1.5	1.7
SA application:						
Control	10.2	10.4	10.2	10.1	10.3	10.1
0.02% SA	10.3	11.2	11.5	11.8	14.3	15.7
0.04% SA	10.4	11.4	11.5	14.3	16.0	17.1
0.06% SA	10.6	11.9	12.0	21.7	25.9	34.1
L.S.D. at (0.05):	n.s.	n.s.	n.s.	1.7	1.8	1.8
Interaction:						
Matabea:						
Control	8.1	8.3	8.1	8.2	8.3	8.1
0.02% SA	8.3	9.8	10.6	11.2	13.1	14.1
0.04% SA	8.3	10.3	10.2	12.7	14.3	14.9
0.06% SA	8.4	10.4	10.6	17.3	22.5	28.8
Mozellae:						
Control	12.4	12.5	12.4	12.1	12.3	12.2
0.02% SA	12.4	12.6	12.4	12.5	15.6	17.4
0.04% SA	12.6	12.6	12.9	15.9	17.8	19.4
0.06% SA	12.8	13.4	13.5	26.2	29.3	39.4
L.S.D. at (0.05):	1.6	1.6	1.8	1.8	1.9	1.9

As Gachomo *et al.* (2003) mentioned that plants have developed remarkable strategies to adapt to environmental changes by using a range of constitutive or inducible biochemical and molecular mechanisms, and many biochemical changes occur in plants after inducers treatments, and some of these have been associated with the expression of related genes, which protect the plant against infection. Several enzymes that appear to be phytoalexin pathway specific have been identified recently, and the genes encoding some of these have been cloned. In pea plants, as Wu *et al.*, (1998) reported, that cDNA encoding the enzyme (+) 6a-hydroxymaachkiain 3-0 methyltransferase involved in the last step of pisatin biosynthesis has been cloned and in Solanaceae and Malvaceae the genes for the first enzyme required for the sequence production has been cloned.

The use of salicylic acid to induce systemic acquired resistance was previously used to minimize the infection with many diseases (Malamy and Kessing, 1992; Dekker, 1996; Sticker *et al.*, 1997; Ibrahim, 1998; Bhatt *et al.*, 1999;Abou-Taleb, 2001; Pradeep and Jambhale, 2002 and Attia, 2004).

Table (6): Total phenols in leaves of sugar peas after treatment with salicylic acid, 2005/06 season.

Treatments	Total phenols (mg/g/dry wt.) Hours after treatment					
	0	24	48	72	96	120
Cultivars :						
Matabea	8.2	9.6	9.8	12.5	13.2	13.9
Mozellae	12.5	12.6	12.7	15.6	17.0	19.4
L.S.D. at (0.05):	1.5	1.5	1.5	1.6	1.6	1.7
SA application:						
Control	10.3	10.3	10.3	10.2	10.2	10.1
0.02% SA	10.4	11.1	11.5	11.6	14.4	15.2
0.04% SA	10.4	11.3	11.6	14.0	16.1	17.1
0.06% SA	10.5	11.8	11.8	20.5	19.8	24.4
L.S.D. at (0.05):	n.s.	n.s.	n.s.	1.8	1.8	1.9
Interaction						
Matabea:						
Control	8.1	8.2	8.2	8.1	8.1	8.1
0.02% SA	8.2	9.7	10.5	10.9	13.1	13.9
0.04% SA	8.3	10.1	10.4	12.3	14.5	14.7
0.06% SA	8.3	10.4	10.3	18.8	17.3	19.1
Mozellae:						
Control	12.5	12.4	12.4	12.3	12.3	12.1
0.02% SA	12.6	12.5	12.5	12.4	15.7	16.5
0.04% SA	12.5	12.6	12.8	15.7	17.7	19.6
0.06% SA	12.7	13.2	13.4	22.3	22.4	29.7
L.S.D. at (0.05):	1.7	1.8	1.8	1.9	1.9	2.0

3. Yield and TSS content:

The reduction in disease incidence resulted in significant increasing in sugar peas yield. Data in Table (7) showed clearly that significant differences were recorded between cultivars in their yield. The resistant cultivar, Mozellae produced 1.398 kg/m^2 compared with 1.224 kg/m^2 produced by the susceptible one i.e., Matabea. Generally, 0.06% SA treatments, significantly resulted in increasing sugar peas yield compared to control treatment, it was 1.458 kg/m^2 with 0.06% SA compared with control plant which produced 1.202 kg/m^2 in 2004/05 season. The same trend was observed due to the interaction effect between cultivars and SA treatments. The susceptible Matabea cv. produced 1.389kg/m^2 compared to control plants which produced 1.082 kg/m^2 under 0.06 SA spraying. The same trend was observed in Mozellae cv. indicating that the systemic acquired resistance is an important component of plant's defense against diseases as well as yield improvement.

Data presented in Table (7) show the effect of SA applications on TSS (%) of pea pods. Results revealed that there were, to some extent, low differences in the estimated TSS due to SA treatments, but no significant differences were recorded. Also, no significant differences were recorded between the susceptible "Matabea" cv. and the resistant one i.e., "Mozellae".

Table (7): Powdery mildew disease incidence, yield and TSS content in sugar peas as affected by salicylic acid.

Treatments	% Disease incidence		Average yield (kg/m²)		T.S.S. (%)	
	2004/5	2005/6	2004/5	2005/6	2004/5	2005/6
Cultivars :						
Matabea	7.0	7.3	1.224	1.198	11.6	11.5
Mozellae	3.2	3.4	1.398	1.369	12.0	12.1
L.S.D. at (0.05):	1.9	1.9	0.053	0.049	n.s.	n.s.
SA application:						
Control	7.1	7.4	1.202	1.199	11.7	11.7
0.02% SA	5.8	6.0	1.273	1.207	11.8	11.8
0.04% SA	5.3	5.4	1.311	1.303	11.8	11.8
0.06% SA	2.3	2.6	1.458	1.427	11.8	11.9
L.S.D. at (0.05):	1.8	1.9	0.059	0.051	n.s.	n.s.
Interaction:						
Matabea:						
Control	9.6	10.1	1.082	1.104	11.5	11.4
0.02% SA	8.1	8.3	1.193	1.097	11.6	11.5
0.04% SA	7.5	7.4	1.234	1.243	11.6	11.7
0.06% SA	3.1	3.5	1.389	1.351	11.7	11.6
Mozellae:						
Control	4.7	4.8	1.323	1.294	11.9	12.1
0.02% SA	3.5	3.8	1.354	1.318	12.0	12.2
0.04% SA	3.1	3.5	1.388	1.363	12.1	12.0
0.06% SA	1.5	1.7	1.527	1.504	12.0	12.3
L.S.D. at (0.05):	1.6	1.7	0.062	0.058	0.5	0.5

CONCLUSION

The present study tested the efficacy of SA treatment in the induction of PAL mediated pathway for pisatin synthesis. SA treatment at 0.06% concentration was able to induce the synthesis of pisatin in the susceptible cultivar, though its value was low when compared with 'Mozellae'. The values for PAL activity and pisatin content were higher in 'Mozellae', which could be the associated with resistance against powdery mildew disease.

Phytoalexins are important defense compounds and future studies on these compounds will continue to provide new insights in to plant-pathogen interactions as well as new approaches to disease control and yield improvement.

REFERENCES

Abo-Taleb, M.A. (2001): Biochemical changes associated with application of some resistance inducing compounds for controlling powdery mildew of cucumber. Egypt. J. Appl.Sci. 16 (12): 387-405.

Agricultural Economic Bulletin, (2005): Annual report, Ministry of Agriculture, Cairo, Egypt

Attia, H.A. (2004): Effect of some traditional and non traditional compounds on controlling strawberry leaf spot and blight disease. Ph.D.Thesis, Fac.of Agric., Cairo Univ.

Beckman, G.H. (2000): Phenolic storing cells: Keys to programmed cell death and periderm formation in wilt disease resistance and in general defense response in plants. Physiol. Plant Pathol. 57: 101-110.

Bhatt, J.C., Dube, S.D. and Chauhan, V.S. (1999): Effect of environmental conditions, salicylic acid and phytohormones on pea leaf blight. Indian Phytopathology., 52(3): 270 - 273.

Burrell, M.M. and Rees, T.A.(1974): Metabolism of phenylalanine and tyrosine by rice leaves infected with *Pyricularia oryzae*. Physiol. Plant Pathol. 4: 497 - 508.

Chen, Z.; Silva, H. and Klessing, D.J. (1993): Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science., 262: 1883 - 1886.

Chondra, A.; Anand, A.; Kumar, P.K. and Saxena, P. (2001): Influence of salicylic acid on protein and catalase activity in relation to systemic acquired resistance in cowpea against root-rot. Indian Phytopathology., 54(3): 284 - 287.

Darvill, A.G. and Albersheim, P.(1984): Phytoalexin and their elicitors - A defense against microbial infection in plants. Annu. Rev. Plant Pathol. 35:243-275.

Dean, R.A. and Kuc, J. (1985): Induced systemic protection in plants. Trend Biotechnol.3: 125 - 128

Dekker, J. (1996): Methionine induced inhibition of powdery mildew and its reversal by folic acid. Crop Protection.15(4): 341 - 347

Dixon, R.A.; Harrison, M.J. and Lamb, C.J.(1994): Early events in the activation of plant defense responses. Annu. Rev. Phytopathology. 32: 479 - 501.

Doubrava, N.; Dean, R.and Kuc, J. (1988): Induction of systemic resistance to anthracnose caused by *Collectrichum lagenarium* in cucumber by oxalates and extracts from spinach and rhubarb leaves. Physiol. Mol. Plant Pathol., 33:60-70

Fouly, H.M. (2004): Effect of some micronutrients and antioxidant salicylic acid on suppressing the infection with strawberry powdery mildew disease. Bull.Fac.Agric., Cairo Univ., 55: 475 -486.

Gachomo, E.W.; Shonukan, O.O. and Kotchoni, S.O.(2003): The molecular initiation and subsequent acquisition of disease resistance in plants. African J. Biotech. 2 (2) : 26 - 32.

Gaffiney, T.; Fridrich, L.; Vernooji, B.; Negotto, D.and Nye, G. (1993): Requirement of salicylic acid for the induction of systemic resistance. Science, 261: 754 - 756

Green, S.K. (1991): (2^{nd} ed.). Guidelines for diagnostic Research and Development Center. Tech. Bul. No. 15.63p

Hammerschmdidt, R. (1999): Phytoalexins: What have we learned after 60 years. Annu. Rev. Plant Pathol. 37: 285-306.

Horsfall, J.G. and Barratt, R.W. (1945):An Improved Grading System for Measuring Plant Diseases. Phytopathology, 55: 655-659.

Ibrahim, F.A. (1998): Induction of resistance to some diseases of cucumber plants grown under greenhouse conditions. Ph.D.Thesis, Fac., Agric., Ain Shams Univ

Karthikeyan, A. and Narayanaswamy, R.(1989): Changes in total phenols in rice varieties inoculated with *Rhizoctonia solani* and treated with carbendazim. Int. Rice Res. Nswl. 14:12-20.

Kessmann, H.; Staub, T.; Hofmann, C.; Meatzke, T.and Herzog, J. (1994): Induction of systemic acquired disease resistance in plants by chemicals. Ann. Rev. Phytopathology., 32: 439 - 459.

Kuc, J. and Rush, J.(1985): Phytoalexins. Arch. Biochem. Biophys. 236: 379 - 389.

Lancke, P. (1981): Alternative chemical agents for controlling plant diseases. Phil. Trans. Res. Soc., (2): 83 - 101.

Linthorst, H. (1991): Pathogensis related proteins of plants. Grit. Rev. Plant Sci., 10: 123 - 150.

Loschke, D.C.; Hadwiger, L.A.; Schroder, J. and Hahkbrock, K.(1981): Effect of light and of *Fusarium solani* on synthesis and activity of phenylalanine ammonia-lyase in peas. Plant Physiol. 68: 680 - 685.

Malamy, J. and Kessing, D.(1992): Salicylic acid and plant diseases resistance. The Plant Journal.20:643-654.

Metreux, J.P. (2001): Systemic acquired resistance and salicylic acid; current state of knowledge. European J. Plant Path. 107: 13-18.

Metreux, J.P. and Boller, T. (1986): Local and systemic induction of Chitinase in cucumber plant in response to fungal, bacteria and viral infection.Physiol.Mol.Pathol., 28:161-169.

Moesta, P. and Grisebach, H. (1982): L-2-aminoxy–3-phenylpropanoic acid inhibits phytoalexin accumulation in soybean with concomitant loss of resistance against *Phytophthora megasperma* f. sp. Glycinea. Physio. Plant Pathol. 21: 65-70.

Munjal, R.L.; Chenulu, V.V. and Hora, T.S. (1963): Assessment of losses due to powdery mildew (*Erysiphe polygoni* DC.) of pea. Indian phytopathology. 16: 268 - 270.

Oku, H.; Ouchi, S.; Shiraishi, T., and Baba, T.(1975): Pisatin production in powdery mildewed seedlings. Phytopathology 65: 1263 - 1267.

Orober, M.; Siegrist, J. and Buchenauer, H.(2002): Mechanism of phosphate-induced disease resistance in cucumber. Eur. J. Plant Path. 108: 345 - 353.

Pradeep, T. and Jambhale, N. (2002): Relationship between phenolics, polyphenol oxidase and peroxidases and resistance to powdery mildew in zizhyphus. Indian Phytopathology.55(2): 195 - 196.

Ragg, H.; Kuhn, D. N. and Hahlbrock, K.(1981): Coordinated regulation of 4-coumarate: CoA ligase and phenylalanine ammonia- lyase mRNA in cultured plant cells. J. Bio. Chem. 256: 10061-10065.

Rusch, H. and Laurence, J. A.(1993): Interactive effects of ozone and powdery mildew on pea seedling. Phytopathology 83: 1298 - 1263.

Salama, H.A. (1990): Enzyme activity as an indicator for micronutrients content in plants. Ph. D. Thesis, Fac. of Agric., Cairo Univ.

Sindhan, G.S. and Parashar, R.D.(1996): Biochemical changes in groundnut leaves due to infection by early and late leaf pathogens. Indian J. Mycol. Plant Pathol. 26: 210-212.

Smith, D.A.; Van Etten, H.D. and Bateman, D.F.(1975): Accumulation of phytoalexins in *Phaseolus vulgaris* hypocotyls following infection by *Rhizoctonia solani*. Physiol. Plant Pathol. 5: 51-64.

Snedecor, G.W. and Cochran, W.G. (1967): Statistical Methods.6th ed. The Iowa State. Univ. Press Ames, Iowa, USA.

Sticker, L.; Mauch-Mani, B. and Metraux, J.P. (1997): Systemic acquired resistance. Annu. Rev. Phytopathology. 35: 235-270.

Swain, T. and Hillis, W.E.(1959): The phenolic constituent of *Prumus domestica*. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10: 63 - 68.

Wu, Q.; Preisig, C.A. and VanEtten, H.D. (1998): Isolation of cDNAs encoding (+) 6a-hydroxymaachiain 3-0-methultransferase, the terminal step for the synthesis of the phytoalexin pisatin in *Pisum sativum*. Plant Mol. Biol. 35: 551 - 560.

تأثير الرش بحمض السالسيليك علي تخليق الفيتوألاكسينات في البسله السكرية وعلاقة ذلك بنسبة الاصابة بمرض البياض الدقيقي والمحصول

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أجريت تجربتان حقليتان في منطقة النوبارية في الموسم الزراعي الشتوي ٢٠٠٤/٢٠٠٥ و ٢٠٠٥/٢٠٠٦، وذلك لدراسة تأثير الرش بحمض السالسيليك المضاد للأكسدة علي تخليق الفيتوألاكسينات في البسلة السكرية، و علاقة ذلك بنسبة الإصابة بمرض البياض الدقيقي والمحصول الناتج، وقد أظهرت النتائج ما يلي:-

- أدي الرش بحمض السالسيليك بتركيز ٠,٠٦% إلي زيادة تركيز الفيتوألاكسين الخاص بالبسلة والمعروف باسم "لبيزاتين"، وذلك بالمقارنة بمعاملات الكونترول أو بالتركيزات الأقل من حمض السالسيليك (٠,٠٢ %، ٠,٠٤%).
- زاد تركيز الفينولات في النباتات المعاملة بالمقارنة بالنباتات التي لم تعامل.
- أدت المعاملات تحت الدراسة إلي زيادة نشاط " إنزيم فينيل الانين أمونيا-ليز" المسئول عن إحداث تخليق "البيزاتين" في النباتات التي تم رشها بحمض السالسيليك وكان الصنف "موزيلا المقاوم أكثر استجابة من الصنف "متابي" الحساس.
- أدي رش النباتات بحمض السالسيليك بتركيز ٠,٠٦% إلي انخفاض نسبة الإصابة بالمرض.
- زاد المحصول في النباتات التي تم رشها بالتركيز ٠,٠٦% بالمقارنة بالتركيزات الاخري او بمعاملات الكونترول .
- لم يلاحظ أي اختلاف معنوي في محتوي القرون من المواد الصلبة الذائبة نتيجة هذه المعاملات.