Inducing Resistance in a Flax Cultivar Susceptible to Rust Disease Caused by *Melampsora lini*

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(Received: 01 April 2013; accepted: 24 May 2013)

Ten resistance elicitors, ammonium tartrate, ascorbic acid, benzoic acid, citric acid, cinnamic acid, hydroquinone, salicylic acid, sodium citrate, sodium metabisulfate and thiourea, were applied as foliar sprays using two methods at concentrations of 200 ppm to evaluate their capabilities to induce resistance in the flax cultivar Sakha 1 against rust disease caused by *Melampsora lini* under greenhouse conditions. The trials were repeated three times. ANOVA indicated that the efficiencies of some resistance elicitors, such as benzoic acid and hydroquinone, were different depending on the application method. Other elicitors, including thiourea, salicylic acid and ammonium tartrate, were highly efficient and were stable in reducing the incidence and severity of flax rust disease. However, ascorbic acid was only moderately effective at reducing the incidence and severity of flax rust.

Key words: Elicitors, Flax, Antioxidant, Fungi, Systemic resistance.

Flax (*Linum usitatissimum* L.), which was cultivated in ancient Egypt and Sumer 10,000 years ago, is considered the oldest and best major fiber crop. It ranks just after cotton in relation to cultivated area and economic importance. Flax is cultivated for both fiber and seed yields[1]. Several diseases threaten flax production. Flax rust is a widespread and devastating disease that quantitatively and qualitatively affects flax production. *Melampsora lini* (Ehren b.) Desm., the causal agent of flax rust, can cause severe losses in seed yield and can reduce fiber quality in flax[2].

Resistant cultivars and/or fungicidal control are the only methods for controlling flax rust[3]. Fungicides are currently one of the most available commercial products for controlling rust diseases. Because breeding for fungal resistance is slow and the use of fungicides is environmentally very risky, alternative control strategies are needed[4]. One potential strategy is the use of compounds that do not have direct antimicrobial activity but do increase resistance, or at least decrease symptoms, in some host-pathogen interactions[5]. Thus, inducing systemic resistance in the host plant has become a good method for minimizing disease incidence/severity at the lowest cost and without environmental pollution[6]. Several compounds could indirectly enhance the induced resistance of plants to different pathogens. The induction of resistance is associated with metabolic and structural changes within the plant. Some chemical compounds, such as salicylic acid (SA), have been successfully used as resistance inducers[6,7].

In addition, various promising chemical compounds have been discovered that act as abiotic inducers and that protect many crop plants against phytopathogens[8-11]. The objective of this

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study was to evaluate a selection of resistance elicitors for their capacity to control flax rust disease and to determine the response of flax plants to foliar applications of these elicitors.

MATERIALS AND METHODS

Pathogen and plant Materials

A mixture of *Melampsora lini* races collected from various flax-producing areas of Egypt was used to infest the susceptible flax cultivar, Sakha 1. Naturally infected plants were used to prepare the inoculum. The Sakha 1 cultivar, which is susceptible to rust disease, was sown in No. 15 pots. The seedlings received normal irrigation and fertilization and were kept under greenhouse conditions (13±2°C and 90% Rh). The seedlings were inoculated after sunset by rubbing healthy leaves with rust-infected field leaves[5]. Treatments were applied to the seedlings immediately before inoculation or 36 h before inoculation. The control plants were sprayed with distilled water only. Ten plants/pot were used 25 days after inoculation for the disease incidence and severity calculations. Plants showing even a single pustule of infection were taken into consideration in the disease incidence. Disease severity was calculated as a percentage of the number of infected leaves out of the total number of leaves on each plant. All trials were conducted in a complete randomized block design with four replicates. The experiments were performed in three periodical experiments Cotton Pathology Department greenhouse, the Plant Pathol. Res. Inst., Agric. Res. Center, Giza.

Chemical elicitors

Ten antioxidant compounds, *i.e.*, ammonium tartrate ($C_4H_{12}N_2O_6$), ascorbic acid ($C_6H_8O_6$), benzoic acid (C_6H_5COOH), citric acid ($C_6H_8O_7.H_2O$), cinnamic acid ($C_6H_5CH=CHCO_2H$), hydroquinone ($C_6H_4(OH)_2$), salicylic acid ($C_7H_6O_3$), sodium citrate ($C_6H_5Na_3O_7.2H_2o$), sodium metabisulfate ($Na_2S_2O_2$) and thiourea (CH_4N_2S), were used. The compounds were dissolved in deionized distilled water (DDW) at concentrations of 200 ppm. DDW was used as a foliar spray control.

Statistical analysis

Analysis of variance (ANOVA) of the data was performed using the MSTAT-C statistical package (a microcomputer statistical program for the design, management and analysis of agronomic research experiments, Michigan State Univ., USA). Least significant difference (LSD) was used to compare treatment mean values.

RESULTS

ANOVA (Table 1) indicated that resistance elicitors (treatment), the method of treatment and

Table 1. Analysis of variance of the effects of treatments (resistance elicitors), spray time and their interactions on flax rust occurrence under greenhouse conditions

Experiments	Source of variation	D.F.	M.S.	F. value	P>F
1st	Replication	3	1.696	1.6120	0.1955
	Spray time (S)	1	1660.833	1578.9815	0.0000
	Treatment (T)	10	399.869	38.1623	0.0000
	T x S	10	92.366	87.8142	0.0000
	Error	63	1.052		
2nd	Replication	3	0.238	0.8519	
	Spray time (S)	1	654.000	2338.0188	0.0000
	Treatment (T)	10	426.140	1523.4292	0.0000
	T x S	10	85.995	307.4275	0.0000
	Error	63	0.280		
3rd	Replication	3	0.654	3.4405	0.1955
	Spray time (S)	1	246.895	1299.5459	0.0000
	Treatment (T)	10	264.172	1390.4857	0.0000
	T x S	10	470.257	248.7412	0.0000
	Error	63	0.190		

their interactions were very highly significant sources of variation over the three periodical experiments. Significant interaction indicates that the effectiveness of some elicitors varied according to the method of application. For example, citric acid and benzoic acid were the most effective in reducing the disease incidence (DI) of flax rust when it was sprayed 36 h before infection compared with the spraying immediately prior to infection (Fig. 1). Other elicitors, such as thiourea, salicylic acid and ammonium tartrate, were effective regardless of the method of treatment. The data in Table 2 suggest that the chemicals reduced disease severity (DS) in the three periodical experiments compared with the control. Although no significant differences were identified in the first experiment between some elicitors when applied just before infection, such treatments showed significant differences when applied 36 h before infection. For example, significant differences were identified between benzoic acid and sodium citrate and between ammonium tartrate and salicylic acid.

Table 2. Effects of treatments (resistance elicitors) and spray time on disease severity of flax rust occurrence under greenhouse conditions

S.	Treatments	1st Experiment		2nd Experiment		3rd Experiment	
No.		A	B	A	A	B	A
1.	Ammonium tartrate	8.2	6.17	6.25	7.87	6.4	5.92
2.	Ascorbic acid	12.67	14.02	12.9	13.65	9.65	10.57
3.	Benzoic acid	22.42	3.60	21.25	5.15	13.77	4.90
4.	Citric acid	25.73	13.57	23.07	10.97	17.32	6.4
5.	Cinnamic acid	14.00	5.52	11.75	8.27	7.85	5.42
6.	Hydroquinone	18.85	6.82	17.35	6.2	10.67	4.05
7.	Salicylic acid	7.67	4.17	8.57	3.45	5.32	2.57
8.	Sodium citrate	22.62	11.52	15.35	17.52	11	13.47
9.	Sodium metabisulfate	27.8	9.55	25.22	12.2	19.65	10.02
10.	Thiourea	2.85	1.17	1.12	0.90	1.22	0.65
11.	Control	30.17	24.45	29.95	26.62	21.15	23.17
	LSD 0.05		1.45		0.75		0.61
	LSD 0.01		1.93		0.99		0.82

Spray times; A= Just before infection, B=36 h before infection

Table 3. Efficacy of treatments (resistance elicitors) and spray time against flax rust occurrence under greenhouse conditions

S.	Treatments	1st Experiment		2nd Experiment		3rd Experiment	
No.		A	B	A	A	B	A
1.	Ammonium tartrate	72.82	74.76	79.13	70.43	69.74	74.45
2.	Ascorbic acid	58	42.66	56.92	48.72	54.37	54.38
3.	Benzoic acid	25.69	85.28	29.05	80.65	34.89	78.85
4.	Citric acid	15.91	44.05	22.97	58.79	18.11	72.38
5.	Cinnamic acid	51.87	77.42	60.77	68.93	62.88	76.61
6.	Hydroquinone	37.52	72.11	42.01	76.71	49.55	82.52
7.	Salicylic acid	74.58	82.94	71.38	87.04	74.84	88.91
8.	Sodium citrate	25.02	52.88	48.74	34.18	48	41.86
9.	Sodium metabisulfate	7.85	60.94	15.79	54.17	7.1	56.75
10.	Thiourea	90.55	95.21	96.26	96.61	94.23	97.19
11.	Control	00.00	00.00	00.00	00.00	00.00	00.00

Spray times; A= Just before infection, B=36 h before infection

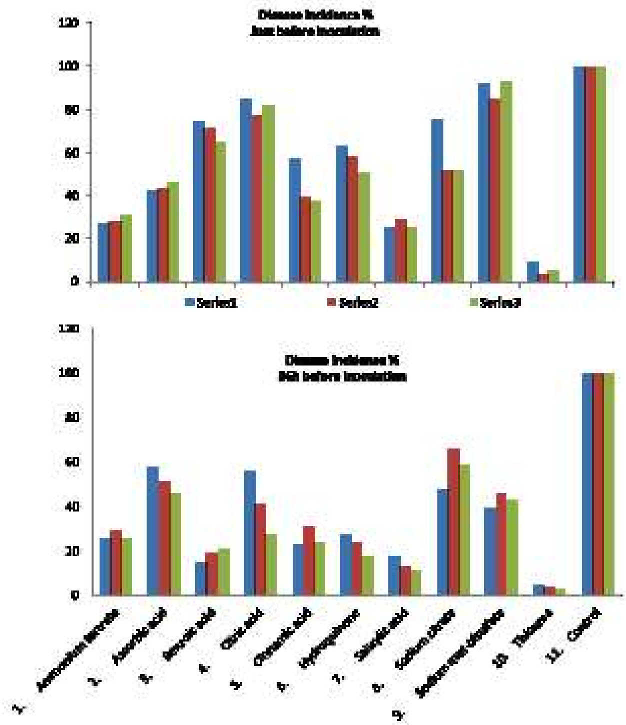

Fig. 1. Effects of treatments (resistance elicitors) and spray time (just before inoculation and 36 h after inoculation) on the incidence of flax rust under greenhouse conditions. (From left to right, series 1= 1st experiment, series 2= 2nd experiment, series 3= 3rd experiment.)

In the second experiment, some elicitors had insignificant effects regardless of the treatment method, such as hydroquinone and sodium citrate. The same results were observed in the third experiment, such as for hydroquinone and ascorbic acid. Some elicitors, such as thiourea and salicylic acid, were highly efficient (71.42% to 97.19%) in reducing rust disease on flax plants in all of the treatments. Other elicitors, such as ammonium tartrate and cinnamic acid, exhibited efficiencies ranging from 51.87% to 79.13% (Table 3). However, benzoic acid and hydroquinone were effective when sprayed 36 h before infection.

DISCUSSION

Because the use of fungicidal controls is very risky, the induction of systemic resistance is an effective and simple approach to disease management. In this study, 10 resistance elicitors were successfully used against flax rust disease and exhibited significant reductions in disease incidence and severity that reached approximately 97% in some treatments. A wide range of chemical compounds has frequently been used to induce systemic resistance in different plants. Ammonium tartrate, ascorbic acid, benzoic acid, citric acid, cinnamic acid, hydroquinone, salicylic acid, sodium citrate, sodium metabisulfate and thiourea have been reported to induce resistance in cotton plants against wilt disease[7,12]. Resistance is induced in many plants by salicylic acid[13], ascorbic acid[10,14,15], citric acid and benzoic acid[8].

Our results indicated that spraying salicylic acid 36 h before infection could induce flax resistance to rust disease, resulting in more than 75% and 90% reductions in disease incidence and severity, respectively. Salicylic acid significantly reduced both the DI and DS of rust disease on sugar beet[5] and reduced the incidences of *Rhizoctonia solani* damping-off and *Macrophomina phaseolina* charcoal rot of sunflower[16]. Moreover, salicylic acid might trigger the resistance of the peanut to seed-borne fungi[17]. The results of this study revealed that thiourea was the most effective inducer of flax resistance to rust disease, resulting in more than 90% and 95% reductions in DI and DS, respectively, when sprayed 36 h before infection. Thiourea has a wide range of uses; for example, it is used an antioxidant[18]. One thiourea derivative compound enhanced resistance against tobacco mosaic virus by the enhancement of defense-related enzymes, such as phenylalanine ammonia lyase (PAL), peroxidase (POD) and superoxide dismutase (SOD), in addition to related activities of defensive enzymes[19].

The effectiveness of some other compounds, *i.e.,* benzoic acid and hydroquinone, varied according to the method of application. The maximum reductions in DI and DS were observed in the group treated 36 h before infection. Seed soaking (12 h before planting) in 20 mM of hydroquinone decreased pre- and post-emergence damping–off [17]. A concentration of 20 mM benzoic acid yielded the lowest values for brown spot DI and DS on rice plants[20].

The efficacies of other compounds were not influenced by application methods; for example, ammonium tartrate exhibited approximately 70%-80% efficacy regardless of the spraying methods. Citric acid and thiourea derivatives have been found to possess many promising biological activities and are used as antibacterial and antifungal agents[21].

The effectiveness of the different elicitors used in this study as inducers of systemic resistance may be due to morphological and/or physiological changes in the host's defense-related compounds such as phenolics and proteins[22,23].

ACKNOWLEDGMENTS

This work was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

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