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Amine Oxidase, Peroxidase, Catalase and Acid Phosphatase Activities in Powdery Mildew Infected Plants of *Cucumis sativus*

By

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K e y w o r d s : *Cucumis sativus, Sphaerotheca fuliginea,* peroxidase, amine oxidase, acid phosphatase, catalase.

S u m m a r y

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The plants of *Cucumis sativus* infected by powdery mildew *Sphaerotheca fuliginea* showed significant increase in the peroxidase activity and extractable protein content five days after the inoculation and about two-fold increase in the amine oxidase activity thirteen days after the inoculation. Changes of the activities of acid phosphatase and catalase in healthy and infected plants were not evident. Histochemical detection of peroxidase confirmed the increased activity of the enzyme that is located in the cell wall and epidermis of infected plants. Expression of three stress-related peroxidase isoenzymes after infection by powdery mildew were observed by native electrophoresis in polyacrylamide gel.

I n t r o d u c t i o n

Powdery mildew is one of the most destructive foliar diseases of cucurbits in both temperate and subtropical climate conditions (SITTERLY 1978). In temperate growing areas, powdery mildew is caused by two fungi from the class

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Ascomycotina, order Erysiphales: *Erysiphe cichoracearum* DC. Ex Mérat and *Sphaerotheca fuliginea* (Schlecht. Ex Fr.) Pollaci (BRAUN 1987).

Research on the defense mechanisms of plants against pathogens has received strong attention in recent years (KOMBRINK & SOMSSICH 1995). Defense responses of plants can be divided according to the nature of the mechanisms involved into structural and biochemical defenses (AGRIOS 1997). Plants respond to the infection in several ways by activating response genes and inducing the formation of their products. Research of the proteins with enzyme activity that take part in the defense mechanism was directed especially to the study of peroxidases (STAEHELIN & al. 1992), superoxide dismutase (LEVINE & al. 1994, SUTHERLAND 1991), callose synthetase (BOWLES 1990, KAUSS 1990, KOHLE & al. 1985, OHANA & al. 1993), lipoxygenase (CROFT & al. 1993) and pathogenesis related proteins (PR proteins) with β-1.3-glucanase (PR-2) (KAUFFMANN & al. 1987) and chitinase (PR-3) activities (LEGRAND & al. 1987).

The main purpose of this study was to compare the changes in enzyme activities (peroxidase, catalase, amine oxidase and acid phosphatase) in powdery mildew infected and non-infected plants of *Cucumis sativus*.

Material and Methods

Plant material

Cucumis sativus (line 6505), obtained from the Breeding Station Smržice, Czech Republic.

Inoculation

Fully expanded cotyledons (7-10 day old) were inoculated by dusting the *Sphaerotheca fuliginea* isolate maintained on cucumber seedlings. Inoculated and control plants were grown in the growth chamber under controlled conditions (18-20 °C, 12 h photoperiod). Plants were harvested daily for 14 days and stored at -20 °C.

Infection degree assessment

Intensity of pathogen sporulation was recorded daily after first occurrence of disease symptoms. Infection degree was assessed on a 0-3 scale (LEBEDA 1986). The mean value of the infection degree was expressed as a percentage of the maximum score.

Preparation of plant extracts

Plant material was homogenized in ratio 1:1 (w/v) with 0.1 M potassium phosphate buffer, pH 7.0, for enzyme activity assay and with 0.1 M Tris/HCl buffer containing 0.1% of 2-mercaptoethanol, pH 7.0, for native PAGE. Extracts were filtered through nylon cloth and centrifuged at 12 000 x g, for 30 min at 4 °C.

Enzyme activity assays

Enzyme activities were assayed by spectrophotometric methods. Amine oxidase activity was measured by the reaction with Ehrlich's reagent (MACHOLÁN & al. 1975), peroxidase activity by a modified method with guaiacol (ANGELINI & al. 1990), acid phosphatase by method with p-nitrophenyl phosphate used as substrate (BERGMEYER & al. 1974), and catalase by assay of hydrogen peroxide based on formation of its stable complex with ammonium molybdenate (GÓTH 1991).

Protein concentration was determined according to Bradford with bovine serum albumin as a standard (BRADFORD 1976).

Histochemical study was performed on cucumber stem slices. Peroxidase activity was localized by activity staining in a mixture of 12.5 ml of 0.1 M potassium phosphate buffer, pH 7.0 and 2.5 ml of cold methanol containing 5 mg 4-chloro-1-naphthol and 20 µl of 26% H_2O_2 for a suitable time period.

Native PAGE

Electrophoretic separations were performed on a slab polyacrylamide gel (10%) with 0.025 M Tris - 0.19 M glycine buffer, pH 8.3, at 4°C. The reaction mixture for staining the peroxidase activity (IMBERTY & al. 1984) contained pyrocatechol, p-phenylenediamine and H_2O_2 in 50 mM Tris/HCl buffer, pH 6.8, and for acid phosphatase activity (TANKSLEY & ORTON 1983) Fast Black K^+ salt and α-naphthyl acid phosphate in 50 mM Na-acetate buffer, pH 5.5.

Results

Peroxidase activity

The first significant increase in the peroxidase activity of the seedlings of *C. sativus* infected by *S. fuliginea* was observed five days after the inoculation when the infection degree reached approx. 25%. A marked increase in the peroxidase activity (Table 1), about 5-fold higher than control values was detected in plants 12 - 14 days after the inoculation when the infection degree was nearly 100% (Table 1). The increase of extractable protein content follows the same pattern (Table 1.).

Table 1. Changes in peroxidase and amine oxidase activities and content of extractable protein in healthy and powdery mildew infected plants of *Cucumis sativus* during infection progress.

Days after inoculation (days)	Peroxidase activity (nkat/ml)		Amine oxidase activity (pkat/ml)		Extractable protein content (mg/ml)		Infection degree (%)
	Healthy plants	Infected plants	Healthy plants	Infected plants	Healthy plants	Infected plants	
1	60	25	16.7	20.8	2.7	2.4	-
2	80	55	25.6	27.0	2.9	2.3	-
3	84	54	15.5	22.1	2.3	1.9	-
4	50	52	16.0	17.8	2.0	1.9	-
5	65	180	18.6	20.0	1.5	2.5	26
7	82	240	15.5	23.0	1.5	2.8	44
8	70	260	18.7	24.2	1.4	3.5	48
9	80	265	21.1	22.8	1.5	3.6	53
10	105	295	24.0	29.2	1.7	3.5	83
11	85	415	17.7	29.7	1.7	4.6	92
12	85	450	23.6	32.2	1.7	4.6	96
13	95	460	20.3	38.3	1.8	4.7	95
14	80	455	18.1	32.8	1.9	4.8	97

for total protein (Fig. 1a) and for peroxidase activity (Fig. 1b). Three major anionic peroxidase isoenzymes appeared on the activity stained patterns. Isoenzyme patterns of peroxidase of infected plants were similar to these of healthy plants, but the intensities were higher.

Histochemical detection of peroxidase confirmed the increased activity of peroxidase located in the cell wall and epidermis of infected plants (Fig. 2).

Amine oxidase activity

Very low amine oxidase activity was found in the seedlings of *C. sativus,* therefore it had to be measured by an ultrasensitive method with the Ehrlich's reagent. About 1.8-fold increase in the amine oxidase activity was observed thirteen days after the inoculation when the infection degree was approx. 100%. Significant increase of the activity was detected seven days after the inoculation at 50% of the infection degree (Tab. 1).

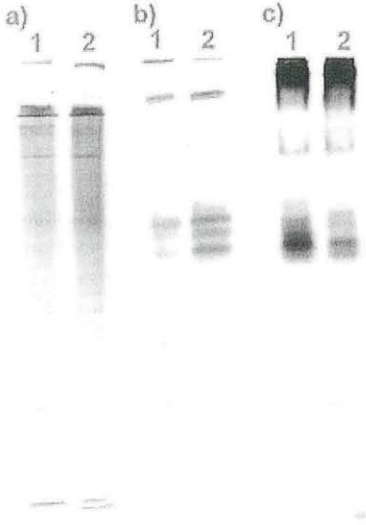

Fig. 1. Extractable protein (a), peroxidase (b) and acid phosphatase (c) activity patterns separated by native PAGE in infected and healthy plants of *Cucumis sativus* 15 days after the inoculation. Each well contains 20 µl of extract: 1) Healthy plant (22.4 µg protein), 2) infected plant (35.7 µg protein).

Native electrophoresis with activity staining of amine oxidase was not successful due to low activity of the enzyme in studied plants.

Catalase and acid phosphatase activities

The changes in activities of catalase and acid phosphatase during the growth of *Cucumis sativus* and after the infection by powdery mildew were not significant. Catalase activity was slightly lower, while the acid phosphatase activity was slightly higher in the infected plants than in control plants. Interestingly a new activity band of acid phosphatase appeared after the infection (Fig. 1c).

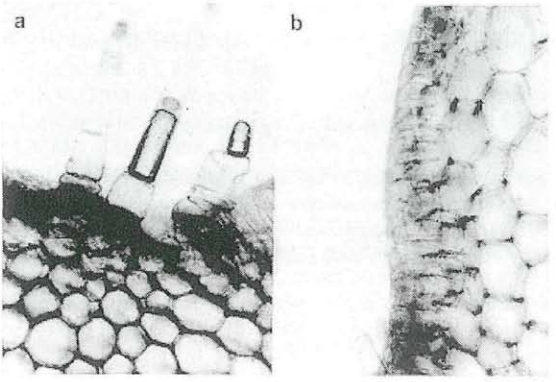

Fig. 2. Histochemical localization of peroxidase in stems of infected (a) and healthy (b) plants of *Cucumis sativus.*

Discussion

From our results it is evident that activities of peroxidases and amine oxidase increase after the infection of *Cucumis sativus* (line 6505) plants by *S. fuliginea*, while acid phosphatase and catalase activities do not show significant changes.

This study follows the recent report on virus induced cucumber peroxidases (REPKA & SLOVÁKOVÁ 1994). In the present study, the existence of three peroxidase izoenzymes which activities were induced by infection of *S. fuliginea* in *C. sativus* (line 6505) was confirmed. Changes of peroxidase activities in the extracts of *C. sativus* (line 6505) infected by *S. fuliginea* are comparable with the results obtained with *C. sativus* (cv. Laura) infected by tobacco necrosis virus (REPKA & SLOVÁKOVÁ 1994). Histochemical localization of the peroxidase in cucumber stems confirms the supposed role of peroxidases in catalyses of the terminal steps in lignin biosynthesis (ANGELINI & al. 1993, GRISEBACH 1981).

Multiple amine oxidases were previously found in cucumber seedlings (PERCIVAL & PURVES 1974). In present study, the activity staining of amine oxidase was not successful from crude extracts of cucumber seedlings due to low

Multiple amine oxidases were previously found in cucumber seedlings (PERCIVAL & PURVES 1974). In present study, the activity staining of amine oxidase was not successful from crude extracts of cucumber seedlings due to low activity, however two activity bands were detected after partial purification of the extract by column chromatography. Activities of amine oxidase in plants of *Cucurbitaceae* are much lower than those in *Leguminosae* (LUHOVÁ & al. 1998) where the activity staining can be done from crude extracts. Increase of amine oxidase activity after the infection of *C. sativus* (line 6505) by *S. fuliginea* (although not so significant as the peroxidase activity increase) corresponds to the data obtained for of chickpea infected by *Ascochyta rabiei* (ANGELINI & al. 1993).

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