Resistance Mechanisms in *Lycopersicon* spp. to Tomato Powdery Mildew (*Oidium neolycopersici*)

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Abstract

Limited information on the resistance mechanisms in *Lycopersicon* spp. to *Oidium neolycopersici* is still available. Macroscopically the resistance is characterized by a very low amount of mycelium development and a lack of sporulation. The leaf surface did not effectively inhibite conidium germination, however significant differences in germ tube and appressorium development were recorded. A large variation was observed in host tissue response. The prevailing resistance mechanism was hypersensitivity (HR). Considerable changes of peroxidase and catalase activities during pathogenesis were detected among tested wild *Lycopersicon* spp. There was positive correlation between increasing of peroxidase activity and extent of necrosis. Histochemistry showed large differences in production of superoxid ions, H_2O_2 and peroxidase in *Lycopersicon* spp. with various level of resistance.

Keywords: *Oidium neolycopersici*; *Lycopersicon* spp.; resistance mechanisms; hypersensitive response; peroxidase; catalase; reactive oxygen species

Although passive or preexisting defence mechanisms could prevent colonization of the plant tissue, more frequently plants activate response to the pathogen attack visually expressed as slowing of pathogen development or plant cell death (hypersensitive response (HR) (HAMMOND-KOSACK & JONES 1996). ROS (reactive oxygen species) have been implicated in numerous developmental and defence responses in plant cells (LOW & MERIDA 1996; LAMB & DIXON 1997). It has been proposed that a rapid increase in either intra or extracellular H_2O_2 is involved in the induction and/or execution of the HR (LEVINE *et al.* 1994; LOW & MERIDA 1996; BESTWICK *et al.* 1997). Various enzyme systems have been proposed to metabolism of ROS. Neutrophil-analogous, membrane-bound NADPH oxidase (LEVINE *et al.* 1994) and apoplastic peroxidase (BOLWELL *et al.* 1995) belong to important enzyme system generating H_2O_2. But peroxidase is also capable to reduce level of H_2O_2 (MONTIES 1989); similarly as catalase which also play the role in catabolism of H_2O_2.

The main purpose of our work was to study infection process of *O. neolycopersici* and tissue responses of *Lycopersicon* spp. (LEBEDA & MIESLEROVÁ 2000) by histochemical and biochemical approaches.

MATERIALS AND METHODS

Plant material. Totally 10 *Lycopersicon* spp. accessions with various level of resistance were used for experiments (Table 1). Pathogen *Oidium neolycopersici*, isolate C/2 (the Czech origin). Inoculation and cultivation followed MIESLEROVÁ *et al.* (2000).

Pathogen development. For visualization of infection structures of the pathogen (6, 24, 48, 72 h and 9 day) the leaf material was stained with cotton blue (after

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clearing in glacial acetic acid – 48 h). Germination of conidia was counted 6, 24 and 48 hpi. The intensity of sporulation of tomato powdery mildew on leaf discs was assessed 9th dpi.

Histological study. Plant responses were recorded as % of infection sites responding by development of necrosis (hypersensitive response), detected as yellow-brownish colouring of cells, under UV excitation autofluorescent (KOGA *et al*. 1988) assessed 24, 48 and 72 hpi.

Histochemical experiments were performed in 4, 8, 12, 16, 20, 24, 48 and 120 h post inoculation of leaves. ***In situ production of*** O_2^- was detected with NBT forming a dark blue insoluble precipitate in the presence of O_2^- (MAY *et al*. 1996). ***In situ production of*** H_2O_2 was detected by the formation of a brownish precipitate after infiltration of leaves under vacuum with 1 mg/ml DAB disolved in water (THORDAL-CHRISTENSEN *et al*. 1997). Control experiments were performed in the presence or absence of 10 mM ascorbate. ***Visualization of POX activities*** were performed in leaves by vacuum infiltration with 200 mM Tris-H-Cl pH 6.0 containing 1 mM DAB and 10 mM H_2O_2. Boiling ethanol was used to stop the reaction and to bleach of the leaves.

Biochemical experiments were performed in 6, 12, 24, 36, 48, 72, 120, 168, 216, 288 and 312 hpi. Enzyme activity was measured by modified method

Table 1. Differences in responses of *Lycopersicon* spp. accessions to *Oidium neolycopersici*

Lycopersicon spp. (genotype/ accession)	Origin	ID* max (%)	Germination (48 hpi) (%)	Mean length of germ tube (48 hpi)(μm)	Sporulation intensity (9 dpi)	% HR 72 hpi	Changes in catalase activity (nkat/mol), 168 hpi	Changes in peroxidase activity (nkat/mol), 168 hpi
L. esculentum cv. Amateur	GBO	100	91	96.5	10^3	0	3.72	30
L. esculentum (OR 4061)	RZ	12.5 (A); 91.3 (J)	83 (A); 97 (J)	34.2 (A); 62.4 (J)	7 (A); 10^2 (J)	43 (A); 2 (J)	180 (J)	170 (J)
L. esculentum (OR 96 000 8)	RZ	50 (A); 90 (J)	81 (A); 96 (J)	40.7 (A); 88.5 (J)	10^1–10^2 (A); 10^2 (J)	12 (A); 2 (J)	–	–
L. chmielewskii (LA 2663)	TGRC	30	79	30.4	10^1–10^2	45	641	155
L. hirsutum (LA 1347)	TGRC	0	68	28.9	0	2	–	–
L. hirsutum (LA 1738)	TGRC	0	70	34.9	0	16	620	290
L. hirsutum f. *glabratum* (LA 2128)	TGRC	0	61	26.7	0	50	180	211
L. parvi orum (LA 1322)	TGRC	48.5	88	78.5	$> 10^2$	40	–	–
L. pennellii (LA 2560)	TGRC	66.3	81	77.9	10^2–10^3	8	–	–
L. peruvianum (LA 445)	TGRC	40	93	84.9	42.5	12	–	–

GBO – Research Institute of Crop Production, Genebank Division, Olomouc, Czech Republic

TGRC – Tomato Genetic Resource Center, University of California, Davis, USA

RZ – Rijk Zwaan, The Netherlands

A – adult; J – juvenile

* percentage of maximum infection degree macroscopically assessed 12th day post inoculation on leaf discs of tested accessions artificially inoculated with *O. neolycopersici* isolate (C-2)

with guaiacol (ANGELINI *et al.* 1990). The catalase was detected by assay of hydrogen peroxide based on formation of its stable complex with ammonium molybdenate (GÓTH 1991).

RESULTS AND DISCUSSION

Data related to the pathogen development revealed that germination of *O. neolycopersici* conidia was not effectively inhibited by host plant. However, in early stages of *O. neolycopersici* infection significant differences in conidia germ tube development on resistant and susceptible accessions were recorded. This is in agreement with some previous results obtained in cereal powdery mildews (AIST & BUSHNELL 1991). Development of mycelia and conidiophores differed from high sporulation intensity on susceptible accessions to non mycelium development on resistant ones (Table 1). Comparable phenomenon was recorded on host and nonhost species of tomato powdery mildew (LEBEDA & MIESLEROVÁ 2000). Significant differences in mycelia and conidiophores development was found in various wild *Lycopersicon* spp. with different level of resistance (MIESLEROVÁ *et al.* 2000).

The most frequent defense response in *Lycopersicon* spp. was hypersensitive reaction (HR), however in some cases followed by pathogen development. On the other hand, the completely resistant accession *L. hirsutum* (LA 1347) showed only limited percentage of cell necrosis per infection site (2%). Nevertheless, our recent data well correspond with conclusions of HUANG *et al.* (1998), who considered HR as a main defence mechanism in this pathosystem. However, he also stressed that HR is not completely responsible for resistance response.

Histochemical experiments showed large differences in production of superoxid ions, H_2O_2 and peroxidase in *Lycopersicon* spp. with various level of resistance. Production of superoxide anion was observed at the first hours post inoculation chiefly on the leaves of highly susceptible accession *L. esculentum* cv. Amateur. Very weak signal only 4–8 hpi was detected in highly resistant accession *L. hirsutum* (LA 1738). Unlike production of O_2^- in this accession the intensive signal H_2O_2 was recorded. In general these results support conclusions of THORDAL-CHRISTENSEN *et al.* (1997) and others who showed that H_2O_2 (substrate for peroxidase) is closely associated with HR.

Considerable changes of peroxidase and catalase activities during pathogenesis were detected. Increased peroxidase (POX) activity was detected in two periods mainly in resistant tomato: 4–12 hpi by histochemical method; 24 hpi and mainly 72–168 hpi by biochemical method. LEBEDA *et al.* (1999) demonstrated that highest increase of POX activity was by moderately resistant accessions with expression of intensive necrotic response. Interestingly, POX activity was detected also inside of *O. neolycopersici*. In this case the role of enzyme can be various; it can be supposed that peroxidase activity localized in apical part of fungus germ tube has important role during penetration of the fungus; on the contrary function of peroxidase in ungerminated conidia (8–120 hpi) is probably related to the pathogen death. The close relationship among changes of peroxidase, ROS generation and hypersensitive reaction of attacked plants was observed, what is known as well as in some other host-pathogen interactions (BESTWICK *et al.* 1997).

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