Role of Plant Cell in Host-Pathogen Interactions: *Lactuca* spp.-*Bremia lactucae*

M. SEDLÁŘOVÁ[1], A. LEBEDA[1], P. BINAROVÁ[2] and L. LUHOVÁ[3]*

[1]Department of Botany, [2]Department of Cell Biology and Genetics, [3]Department of Biochemistry, Faculty of Science, Palacký University, 783 71 Olomouc-Holice, Czech Republic

[*]E-mail: lebeda@prfholnt.upol.cz

Abstract

Reactions of *Lactuca* spp. genotypes with different mechanisms of compatibility/incompatibility to *B. lactucae* race NL16 were examined. Microscopical study revealed significance of initial stages of infection for establishment of the host-pathogen relation. Incompatibility to the pathogen race is mostly expressed as hypersensitive reaction (HR). Rearrangement of cytoskeleton can participate in blocking of fungus penetration in resistant genotypes as well as support development of fungal infection structures in susceptible ones. During infection process peroxidase is activated, H_2O_2 released and phenolic compounds deposited. These defence processes well correspond with the expression of resistance. On the other hand, formation of callose attending pathogenesis is not directly related to incompatibility.

Keywords: *Bremia lactucae*; *Lactuca* spp.; hypersensitive response; reactive oxygen species; cytoskeleton; phenolic compounds; defence mechanisms; histochemistry

INTRODUCTION

Study of cell invasion by biotrophic fungal parasites, including processes elicited during hypersensitive response (HR) in gene-for-gene based interactions bear for valuable features of pathogenesis (HEATH 1999). HR, prevailing mechanism of race-specific defence, rarely occurs also in non-host interactions or some compatible interactions (LEBEDA *et al.* 2001). Significance of host cytoskeleton and phenolic compounds in the restriction of pathogen development in interactions of lettuce (*L. sativa*), wild close relatives from *Lactuca* genus and lettuce downy mildew (*B. lactucae*) was confirmed (SEDLÁŘOVÁ *et al.* 2001; SEDLÁŘOVÁ & LEBEDA 2001b). Cytoskeleton acts in host recognition of pathogen/non-pathogen, its gathering retards penetration as well as further fungal growth in resistant genotypes. Cytoskeleton dynamics is involved in intracellular transport and fortification of cell wall. Its disintegration during localized host cell death is attended by accumulation of autofluorescent phenolic compounds. Importance of other defence mechanisms was proposed, e.g. phytoalexins and phenolic compounds (MANSFIELD *et al.* 1997). We wondered the role of oxidative stress. Active oxygen species are known to play several roles in defence of plants (VAN BREUSEGEM 2001): direct antimicrobial action (e.g. *L. sativa*-*Pseudomonas syringae* pv. *phaseolicola*) (BESTWICK *et al.* 1997); as secondary messengers to be responsible for activating of genes involved in biosynthesis of PR-proteins, phytoalexins, phenolics (MANSFIELD *et al.* 1997); to promote lignification (not found in *Lactuca* spp. – BENNETT *et al.* 1996). Our task was to detect sites of peroxidase activity, H_2O_2 generation and get knowledge whether their location corresponds with deposition of phenolics and pattern of cytoskeleton rearrangement.

MATERIALS AND METHODS

Plant material. Nine genotypes of *Lactuca sativa* L., *L. serriola* L., *L. saligna* L., *L. virosa* L. (Table 1). Pathogen *Bremia lactucae* Regel, race NL16 (Avr14+ Avr15+ Avr18). Inoculation and cultivation

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followed SEDLÁŘOVÁ and LEBEDA (2001a). Detection of hydrogen peroxide (H_2O_2). Leaves were stained with 1 mg/1 ml aqueous solution of DAB (diaminobenzidine) for 5 h. For controls, staining solution supplemented with antioxidant (10mM ascorbic acid). Detection of peroxidase. Solution of 1 mM DAB and 10mM H_2O_2 in 200 mM Tris-HCl buffer, pH 6.0 for 5 h. In each case leaves were cleared in boiling 96% ethanol for 15´ after labelling (THORDAL-CHRISTENSEN *et al.* 1997).

RESULTS AND DISCUSSION

During first hours after inoculation (6–12 h ai) a prompt rise in peroxidase activity was detected as brown-reddish coloration of leaf veins in resistant genotypes. In some susceptible plants (e.g. *L. sativa* UCDM2) weaker labelling was detected as well. High peroxidase activity was detected during pathogen development in germinating spores, germ tubes, appressoria and penetration pegs. Signal highly localized to penetrated cell, rarely also to several neighbouring cells (esp. *L. virosa*), was found later (from 18–24 hai) in high frequency in resistant genotypes. Its occurrence in compatible genotypes was sporadic and weaker, however, it was determined.

Sites of H_2O_2 accumulation co-localized with the signal for peroxidase near cell wall, in periplasmic space. Sometimes a signal expanded to lower layers of tissue (intercellular spaces of mesophyll). In several cases intensive staining colorized the plasma membrane invaginated by forming primary vesicle. Intensity of staining for studied genotypes is summarized in Table 1 and compared to other defence mechanisms. More precise study of this phenomen is in progress.

Timing and localization of oxidative processes corresponded to previously studied phenotypic reaction, occurrence of HR and phenolic compounds release in given genotypes (SEDLÁŘOVÁ & LEBEDA 2001a,b). Sites of peroxidase activity were those where hydrogen peroxide was accumulated, for second one moreover intercellular localization was confirmed. As no lignification was observed in defence to *B. lactucae* (BENNETT *et al.* 1996), ROS action could be suggested for: (1) signal transduction (staining in veins, infrequently in spots behind attached spores), (2) early phenols accumulation (in accordance with

Table 1. Characteristics of *Lactuca* spp. accessions reaction to *Bremia lactucae* race NL16

Lactuca spp. genotype (cultivar/accession)	Resistance genes	Type of resistance	Reaction phenotype to race NL16	N/IS (48 hai)	Subepidermal necrosis	Cytoskeleton rearrangement	Deposition of phenols	Accumulation of H_2O_2
Lactuca sativa								
Cobham Green	R?	RS	compatible	0.10	–	B	–/+	–/+
UCDM 2	*Dm2*	RS	compatible	0.12	-	B,MP	+	+
Mariska	R18	RS	incompatible	0.47	–	B,MP,DAP	+++	++
Lactuca serriola								
LSE/18	*Dm16*	RS	compatible	0.19	–	B	–	–/+
PIVT 1309	*Dm15*	RS	incompatible	1.00	+	B,MP,DAP	0	+++
Lactuca saligna								
CGN 05147	R?	RS	incompatible	1.00	+	G,B,DAP	0	++
CGN 05271	R39	R*	incompatible	0.19	–	G,B,DAP	–	++
Lactuca virosa								
CGN 04683	R?	RS	incompatible	0.56	++	G,MC,DAP	0	+++
NVRS 10.001 602	R?	RS	incompatible	1.00	–	G,MC,DAP	+++	++

RS = race-specific resistance, R* = no effective RS (non-host?), N/IS proportion of necrosis per infection site

Reorganization of cytoskeleton: G microtubules gathered under appressoria, B basket formed by microtubules and microfilaments surrounding primary infection structures, MP microtubular patches, MC microtubular cables, DAP depolymerization of cytoskeleton and generation of auto uorescent phenolic compounds

Degree of signal: – not present, + weak, ++ moderate, +++ intensive staining, 0 data not available intracellular colocalization of H_2O_2 and phenols near cell plasma membrane), (3) oxidative burst during HR (H_2O_2 accumulation in mesophyll bounded to rapid and extensive HR, frequently with subepidermal necrosis (SEN), in *L. virosa* genotypes). Staining for peroxidase and H_2O_2 in cells of susceptible genotypes in some cases later during pathogenesis could be also explained by HR (LEBEDA *et al.* 2001).

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