Cellular mechanisms involved in the expression of specificity in *Lactuca* spp. - *Bremia lactucae* interactions

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Abstract: *Lactuca* spp.-*Bremia lactucae* pathosystem includes very wide range of different host-pathogen interactions and resistance mechanisms. Aspects of expression of these resistance mechanisms have been studied in lettuce (*Lactuca sativa*) and related wild species (*L. serriola, L. saligna, L. virosa*). The role and relationships among early stages of pathogen development and active host defence responses – hypersensitive reaction (HR), cytoskeleton rearrangement, generation of reactive oxygen species (ROS) and phenolic compounds deposition are discussed.

Keywords: lettuce, lettuce downy mildew, defence mechanisms, reaction timing, hypersensitive response, cytoskeleton, phenolic compounds, reactive oxygen species, histo- and immunocytochemistry

Introduction

It has previously been shown that there are at least four categories of resistance mechanisms (non-host, race-specific, race-nonspecific, field) in interactions between *Lactuca* spp. - *Bremia lactucae* (Lebeda et al., 2001b). It is also well known that the crucial events responsible for host-pathogen recognition occur in the first stages of pathogenesis (Lebeda and Schwinn, 1994; Lebeda et al, 2001a,b). Incompatible host plants carrying specific genes for resistance exhibit a sequence of distinct defence events leading to pathogen arrest (Hammond-Kosack et al., 1997; Shirasu and Schulze-Lefert, 2000; Lebeda et al., 2002; Niks and Rubiales, 2002). Within various *Lactuca* spp. - *B. lactucae* interactions both race-specificity and race-nonspecificity occur, characterized by substantial variability in host reaction to pathogen infection exhibited at phenotypic, tissue and cellular level (Lebeda and Reinink, 1994; Mansfield et al., 1997; Lebeda and Pink, 1998; Lebeda et al., 2001b, 2002; Sedlářová et al., 2001a). A knowledge of these mechanisms could be useful to diversify the deployment of resistance genes to genetically protect crops (Niks and Rubiales, 2002).

In this paper, we consider the various defence barriers and responses that occur during the initial stages of the infection process of the oomycete and biotrophic parasite *B. lactucae* on *Lactuca* spp. genotypes with different resistance mechanisms and genetical background.

Processes and mechanisms involved in the expression of host-pathogen specificity

Development of pathogen infection structures

Microscopic studies revealed the significance of the initial stages of infection by *B. lactucae* for the determination and establishment of the host-pathogen relationship. Within 48 hai (hours after inoculation) considerable differences in pathogen development distinguish compatible from incompatible interactions (Table 1). Prompt growth and early formation of primary infection structures (germination and germ tube length, appressoria, primary and secondary vesicles) leading afterwards to colonisation, is typical for susceptible plants,

whereas pathogen growth retardation and/or complete arrest occurs in resistant plant genotypes. However, there are substantial differences in these processes among different resistance mechanisms and within them (Lebeda et al., 2001b, 2002; Sedlářová et al., 2001a).

Table 2. The expected role of the plant cytoskeleton in host-pathogen interactions, compiled according to Škalamera and Heath (1998); Reichel et al. (1999); Sedlářová et al. (2001).

Feature	Role	Cytoskeleton component
Signalling pathways	Recognition of a pathogen/nonpathogen	MFs (MTs)
Resistance responses	Cytoplasmic aggregation	MFs
Intercellular transport	Nuclear and organelle movements,	MFs, MTs
	distribution of phenolic compounds,	MTs
	hypersensitive cell death	MTs (MFs)
Intracellular transport	Virus protein diffusion via plasmodesmas	MTs

MTs – microtubules, MFs - microfilaments

Cytoskeleton

The cytoskeleton is partially responsible for the cell shape, but it is also vital for many of the interconnections of the cell, as well as for cell movement and division. Its reorganization is one of the first plant responses to pathogen attack, being involved in sensing of pathogen/ non-pathogen, various defence mechanisms or allowing pathogen spread within host tissue (Škalamera and Heath, 1998; Reichel et al, 1999) (Table 2).

The process of cell invasion by *B. lactucae* influences the host cytoskeleton pattern and causes its rearrangement in a specific way. It can participate in blocking of fungus penetration in resistant genotypes as well as support development of infection structures in susceptible ones. The degree of changes is tightly associated with the stage of pathogenesis and specific gene combinations. The organization of microtubules (MTs) and microfilaments (MFs) is controlled developmentally (Table 3). Actin filaments are destroyed at an early stage and fungal attack leads to their depolymerization in both susceptible and resistant plants. In resistant plants cortical microtubules gather under maturing appressoria and penetration hypha, and later (during formation of primary and secondary vesicles) they reorientate and form a microtubular basket. Aggregation of MTs may favour deposition of callose (Sedlářová and Lebeda, 2001b).

Phenolic compounds

Accumulation of phenols around primary infection structures and near the cell wall of the infected host cell is a specific feature of incompatibility in race-specific interactions of *L. sativa* and *L. virosa*; whereas callose deposition is greater in compatible interactions and generally in genotypes of *L. sativa*. Thus it is not expected to be primarily involved in defence (Sedlářová and Lebeda, 2001b). Accumulation of autofluorescent phenolic compounds is associated with initiation of a hypersensitive reaction (HR) and occurs with cytoskeleton destruction. MTs were proposed to act in distribution of vesicles with phenolic compounds along the cell wall, however, direct experimental evidence is still lacking (Sedlářová et al., 2001). Besides HR being the prevailing defence mechanism, phenols and phytoalexins were reported as well to act in the process of pathogen inhibition (Mansfield et al., 1997).

Table 3. Changes in microtubular organization of epidermal cells in *Lactuca* spp. in relation to the stages of infection process of *B. lactucae*.

1. Appressorium formation and penetration
Cells of resistant genotypes
Cortical MTs gather under maturing appressoria and penetration hyphae
„Cables“ - thick MTs bundles in penetrated cells
Cells of susceptible genotypes
No detectable changes compared to intact (control) plants
2. Formation of primary and secondary vesicles
MT „basket“ surrounding primary infection structures
3. Hypersensitive response (necrotic cells)
MT „patches“ – signal in spots
Depolymerization of MTs, generation of autofluorescent phenolics, signal within all cells

Reactive Oxygen Species (ROS)

Co-products of regular cellular metabolisms, ROS (Reactive Oxygen Species), are generated at high levels under stress conditions. To protect themselves against their harmful action, plants have developed ROS-scavenging system including enzymes eliminating superoxide (superoxide dismutases) and hydrogen peroxide (catalases and peroxidases) (Lebeda et al., 2001a). ROS are also utilized in a beneficial way, e.g. for induction of defence mechanisms upon infection (Van Breusegem et al., 2001). Several roles in lettuce defence against pathogens have been proposed for ROS: direct antimicrobial action (Bestwick et al., 1997); as secondary messengers being responsible for activating genes involved in biosynthesis of PR-proteins, phenolics and phytoalexins (Mansfield et al., 1997); promoting cross-linking of lignin precursors and cell wall proteins (lignification is not present in *Lactuca* spp.; Bennet et al. (1996)); favouring HR (Bestwick et al., 2001).

The objective of our work was to detect sites of peroxidase activity, $O^{\cdot -}_2$, H_2O_2 generation and to obtain information whether their location corresponds with deposition of phenolics and the pattern of cytoskeleton rearrangement. Sites of $O^{\cdot -}_2$ and H_2O_2 production were localized cytochemically (Mellersh et al, 2002) in leaf tissue infected by the pathogen (Table 1). Increase of H_2O_2 level is timed early after pathogen penetration and is probably connected to signalling within plant cells. H_2O_2 is accumulated in the periplasmic space both in compatible and incompatible interactions, being much more intensive in the later. A large oxidative burst follows in the later stages of infection and this is responsible for cell death (HR). Sites of H_2O_2 accumulation are co-localized with the signal detected for peroxidase near the cell wall, in the periplasmic space. Sometimes a signal in the lower layers of tissue occurred (intercellular space of mesophyll tissue). In some cases intensive staining occurred in the plasma membrane invaginated by a primary vesicle. However, more precise study of this phenomenon would be required to confirm the localization.

High peroxidase activity was localized in developing appressoria and during penetration. Increase in activity of enzymes (peroxidase, superoxid dismutase and catalase) involved in metabolism of ROS is currently being studied. Differences in timing and extent of reactions are expected to be tightly related to the process of cell death (e.g. very fast in *L. virosa*; Lebeda and Pink (1998)). Generation of ROS is considered to be primarily responsible for HR elicited by the pathogen. Rarely the HR (mostly a race-specific defence mechanism) is exhibited in compatible interactions or non-host resistance (Lebeda et al., 2001b, 2002).

Hypersensitive reaction

Incompatible host-pathogen interactions are characterized by HR - a 'suicide' of several host cells at the site of pathogen attack limiting its further spread. The process of programmed cell death (PCD) is controlled genetically, its expression can be prehaustorial or posthaustorial depending on the gene-for-gene combinations (Niks and Rubiales, 2002). Changes to homeostasis of H_2O_2 and $O^{\bullet-}_2$ have been found essential for HR induction by initiating transduction pathways.

In wild *Lactuca* spp. the frequency of HR is mostly correlated with its extent and expression in the later stages of interaction (Table 1). In incompatible genotypes of *L. sativa*, the number of cells per necrotic spot (infection site) never exceeded one. However, a low frequency of HR also occurred in compatible interactions (Lebeda et al., 2001b). In *L. saligna* the development of the pathogen was reduced (extremely in CGN 05271), no intercellular hyphae and virtually no haustoria were formed 48 hai. Formation of primary vesicles in *L. saligna* (CGN 05147) ceased early after penetration with frequent expression of HR However, in CGN 05271 an effective resistance mechanism, distinct from HR, was found (Lebeda and Reinink, 1994; Sedlářová and Lebeda, 2001a). Recently, in CGN 05271 was described a new gene R39, genetically showing features similar to *Dm* genes (Jeuken and Lindhout, 2002).

Conclusions

During the early stages (24-48 hai) of the infection process of *B. lactucae* peroxidase is activated, H_2O_2 released and phenolic compounds are deposited. Initiation of these processes as well as reorganization of cytoskeletal components is directly related to incompatibility and corresponds well with the phenotypic expression of resistance. HR, is the prevailing mechanism of race-specific response. It also rarely occurs in non-host responses or some compatible interactions (Lebeda et al., 2001a,b, 2002). The significance of the host cytoskeleton and phenolic compounds in the arrest of *B. lactucae* development was confirmed (Sedlářová et al., 2001; Sedlářová and Lebeda, 2001b). The cytoskeleton acts in recognition of pathogen/non-pathogen, its reorganisation plays a role in retarding penetration, as well as further fungal growth. The dynamics of the cytoskeleton plays a role in intracellular transport and fortification of cell walls. Its disintegration during HR is accompanied by accumulation of autofluorescent phenolic compounds.

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Table 1. Characteristics of *Lactuca* spp. response to *B. lactucae* race NL16 (compiled according to Sedlářová and Lebeda (2001a,b); Sedlářová et al. (2001)).

Lactuca spp. genotype	R-genes	Type of resistance	SD 14 dai	Reaction phenotype to race NL16	Occurrence of first PV(h)	N/IS[1]	Reorganization of cytoskeleton[1]	Deposition of phenols[1]	Deposition of callose	Accumulation of H_2O_2[1]
Lactuca sativa										
Cobham Green	R?	RS	84,2±8,2	compatible	12	0,10	B	-/+	+++	-/+
UCDM 2	*Dm*2	RS	86,7±1,2	compatible	24	0,12	B,MP	+	++	+
Mariska	R18	RS	0	incompatible	24	0,47	B,MP,DAP	+++	++	++
Lactuca serriola										
LSE/18	*Dm*16	RS	53,3±7,2	compatible	12	0,19	B	-	+++	-/+
PIVT 1309	*Dm*15	RS	1,7±4,0	incompatible	12	1[SN]	B,MP,DAP	0	0	+++
Lactuca saligna										
CGN 05147	R?	RS	0	incompatible	24	1[SN]	G,B,DAP	0	0	++
CGN 05271	R39	RS	0	incompatible	24	0,19	G,B,DAP	-	+	++
Lactuca virosa										
CGN 04683	R?	RS	0	incompatible	36	0,56[SN]	G,MC,DAP	0	0	+++
NVRS 10.001 602	R?	RS	0	incompatible	24	1	G,MC,DAP	+++	+	++

R? = unspecified resistance factor (gene), RS = race-specific resistance, SD = sporulation degree, N/IS = proportion of necrosis per infection site, PV = primary vesicle, h = hours after inoculation, [SN] = subepidermal necrosis

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 autofluorescent phenolic compounds

[1] data obtained 48 hours after inoculation

Degree of signal: - = not present, + = weak, ++ = moderate, +++ = intensive staining, 0 = data not available