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REVIEW

What can we learn from clubroots: alterations in host roots and hormone homeostasis caused by *Plasmodiophora brassicae*

Jutta Ludwig-Müller · Astrid Schuller

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Abstract The clubroot disease of cruciferous crops is caused by an obligate biotrophic protist, *Plasmodiophora brassicae*. The disease is characterized by the development of large root galls accompanied by changes in source-sink relations and the hormonal balance within the plant. Since the disease is difficult to control, it is of high economic interest to understand the events leading to gall formation. In this review we will give an overview on the current knowledge of changes brought about in the host root by this obligate biotrophic pathogen. Emphasis will be on the regulation of changes in plant hormone homeostasis, mainly auxins and cytokinins; the possible role of secondary metabolites, especially indole glucosinolates, in gall formation and auxin homeostasis will be discussed. Also, results from mutant analysis and microarrays using the model plant *Arabidopsis thaliana* are presented.

Keywords *Arabidopsis thaliana* · Biotrophic protist · Brassicaceae · Clubroot disease · Plant hormones

J. Ludwig-Müller (✉) · A. Schuller
Institut für Botanik, Technische Universität Dresden, 01062 Dresden, Germany
e-mail: Jutta.Ludwig-Mueller@tu-dresden.de

Introduction

Obligate biotrophic plant pathogens like *Plasmodiophora brassicae* establish an intricate interaction with their host during at least some parts of the infection process. Their dependence on host carbon sources is obvious but they might also be connected to host metabolism with respect to additional compounds like organic nitrogen, vitamins and minerals. They influence host physiology and alter host regulatory networks.

Clubroot disease development is restricted mainly to members of the mustard family and to a few other plants (Ludwig-Müller et al. 1999b), although *P. brassicae* is capable of infecting the root hairs of several non-cruciferous hosts in Gramineae, Rosaceae, Papaveraceae, Polygonaceae, Resedaceae, and Leguminosae (Webb 1949; MacFarlane 1952). In addition to crop plants, it is known that also wild crucifer species like *Capsella bursa-pastoris* (Buczacki and Ockendon 1979), *Cardamine* sp. (Tanaka et al. 1993), and *Arabidopsis thaliana* (Mithen and Magrath 1992) present on field plots can be infected with the clubroot pathogen.

This plant disease is one of the most devastating within the family of Brassicaceae, and the disease is difficult to control by either chemical or cultural means (Crisp et al. 1989). Clubroot-infected plants are usually dwarfed compared to healthy plants and when such plants are pulled out of the soil, the root system shows typical gall formation (Fig. 1). At maturity, the

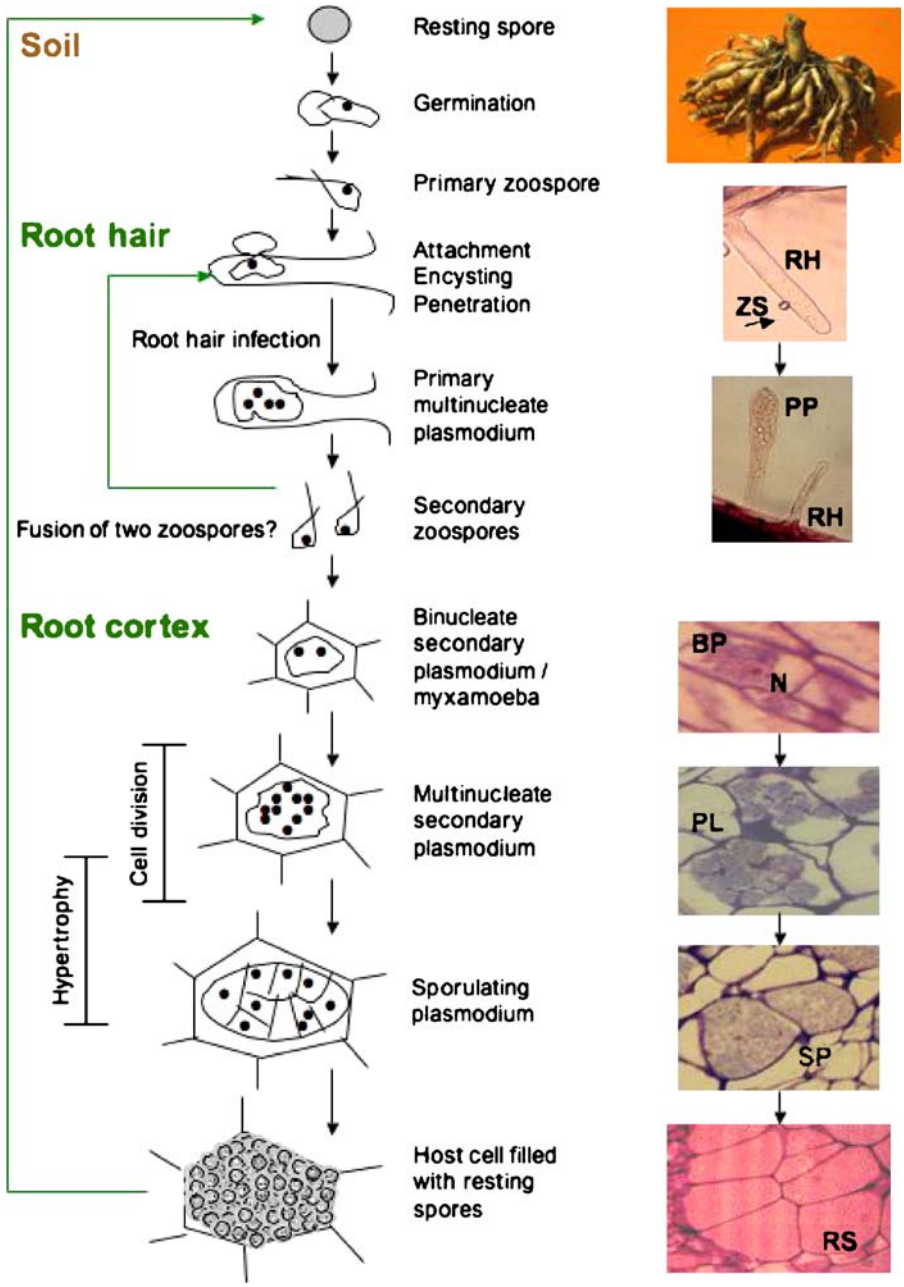

Fig. 1 The life-cycle of *Plasmodiophora brassicae* (*left panel*) illustrated by microscopic pictures of characteristic developmental stages of the pathogen (*right panel*) and a mature root gall of *Brassica* sp. The developmental stages during which cell divisions and hypertrophy occur are marked. A detailed description is given in the text. Abbreviations as they appear from the top of right panel: *ZS* zoospore (*arrow*), *RH* root hair, *PP* multinucleate primary plasmodium, *BP* binucleate secondary plasmodium (myxamoeba), *N* host nucleus, *PL* multinucleate secondary plasmodium, *SP* sporulating plasmodium, *RS* resting spores. Pictures were taken by Claudia Seidel and Jutta Ludwig-Müller

galls turn brown and a large portion of the infected roots remains under ground when the plants are harvested. Thereby the spores are liberated from the plant tissue and can remain infectious for up to 15 years (Mattusch 1977). Traditional control of clubroot includes raising the pH of the soil and rotating susceptible crops with non-cruciferous crops (Webster and Dixon 1991a; b). Growing resistant *Brassica* cultivars is another widely used alternative (Hirai 2006).

Two ecotypes of the model plant *A. thaliana* were identified, which showed a clear incompatible interaction with a distinct *P. brassicae* isolate (Fuchs and Sacristan 1996). The resistance reaction was accompanied by a hypersensitive response in roots. Infected cells were surrounded by necrotic boundaries, which restricted growth of the pathogen (Kobelt et al. 2000). These resistant ecotypes were characterized by the absence of typical clubroot symptoms, a slight reduction in number of lateral roots and the occurrence of

host cell necrosis, which were macroscopically visible as brown spots. Resistance to *P. brassicae* in these ecotypes is conferred by a dominant allele of a single nuclear gene. In contrast, genetic analysis of resistance to clubroot in *B. oleracea* has indicated that it is multigenic (Voorrips and Kanne 1997; Hirai 2006). Once *Brassica* cultivars bred for resistance are in the field for several years, the resistance may be overcome by new virulent *P. brassicae* strains (Mattusch 1994). This is only one example of differences occurring between clubroot infection in *Brassica* spp. and *Arabidopsis*. Others that concern hormone homeostasis will be discussed below.

Short introduction to the life-cycle and biology of the clubroot pathogen

The employment of molecular systematics indicated that *P. brassicae* belongs to the kingdom of Protista (Margulis et al. 1989) where it was grouped in the phylum Cercozoa (Cavalier-Smith and Chao 1997, 2003; Bulman et al. 2001). Together with other plant pathogens of the genus *Polymyxa* and *Spongospora*, *Plasmodiophora* comprises the order of the Plasmodiophorida (Cavalier-Smith and Chao 1997; Bulman et al. 2001). These have been considered as a monophyletic group based on: (1) an unusual form of nuclear division called cruciform division, (2) the presence of biflagellated, heterocont zoospores, (3) the presence of multinucleate plasmodia, (4) environmentally-resistant resting spores, and (5) obligate intracellular parasitism (Braselton 1995). This taxonomic unit was confirmed based on molecular systematic evidence using, for example, actin and ubiquitin sequences (Archibald and Keeling 2004; Bass et al. 2005) and SSU rRNA (Bulman et al. 2001).

Despite the fact that *P. brassicae* was identified as the causal agent of clubroot disease at the end of the nineteenth century (Woronin 1878), its life-cycle is still not entirely clear. The life-cycle of *P. brassicae* consists of two phases (Fig. 1): the primary phase, which is restricted to root hairs and epidermal cells of the host, and the secondary phase which occurs in the cortex and stele of roots and hypocotyl and leads to abnormal development (Ingram and Tommerup 1972).

When the haploid resting spores reach the vicinity of host roots, they start to germinate and primary zoospores are released. These zoospores become attached to a root hair opposite the point of flagellar insertion (Aist and Williams 1971) thereby starting the primary infection phase. The parasite forms a cyst which produces a tubular structure (rohr) containing a projectile-like structure (stachel), which is used to penetrate the host cell wall (reviewed in Braselton 1995). After formation of an adhesorium the parasite is injected into the root hair by the stachel where it appears as a small spherical amoeba. During further development the amoeba enlarges and several nuclear divisions occur (Williams et al. 1971) leading to the formation of a primary multinucleate plasmodium. This plasmodium develops through cleavage into zoosporangia and the development of the pathogen during the first infection cycle is completed with the release of 4–16 uninucleate zoospores to the exterior (Ayers 1944; Ingram and Tommerup 1972). Currently, the release of the secondary zoospores into the soil, and the fusion of the secondary zoospores prior to the infection of the root cortex that apparently occurs, is not resolved in detail. However, since it was possible to inoculate plants with single resting spores leading to club formation, it is assumed that for successful infection and symptom development, different mating types of *P. brassicae* are not required (Narisawa et al. 1996).

Once the pathogen has penetrated the cortex, secondary infection starts with the distribution of the pathogen in the form of a binucleate secondary plasmodium (myxamoeba) within the host tissue. However, the mode of distribution is not yet entirely clear (Buczacki 1983; Mithen and Magrath 1992; Kobelt 2000). According to Ingram and Tommerup (1972) mitotic divisions of the plasmodial nuclei occur in the binucleate secondary plasmodium and multinucleate secondary plasmodia are formed; these were observed in the central stele and were localized in the cells of the cambium and phloem parenchyma (Ludwig-Müller et al. 1999a; b; Kobelt 2000). This stage is accompanied by pronounced cell divisions and hypertrophic cells are formed. During further pathogen development cleavage of the plasmodium results in the formation of numerous resting spores. Although meiosis occurs during the cleavage of sporulating plasmodia into resting spores, it is still not known at which point in the life cycle karyogamy takes place (Braselton 1995).

Despite many efforts it has not been possible to cultivate the pathogen outside its host (Arnold et al.

1996). Several defined in vitro systems such as callus (Dekhuijzen 1975; 1981), hairy root cultures (Graveland et al. 1992; Asano et al. 2006) or suspension cultures (Asano and Kageyama 2006) have been described, but there is virtually no growth and no offspring of *P. brassicae*, so that these systems cannot replace inoculation in the soil for gall formation. Due to its obligate parasitism, information on physiological processes in *P. brassicae* is very scarce. The first sequence coding for an mRNA of *P. brassicae* induced *in planta* was reported by Ito et al. (1999), but after database searches it was difficult to assign a function to this mRNA. In contrast, the *PbTPS1* gene of *P. brassicae*, which was also expressed *in planta*, was identified as a trehalose-6-phosphate synthase (TPS) gene, based on the high homology of the predicted protein sequence to other TPS proteins (Brodmann et al. 2002). The molecular cloning of the *PbSTKL1* gene from *P. brassicae* with homology to kinase revealed another gene with a possible function (Ando et al. 2006b). Bulman et al. (2006) isolated 76 ESTs of *P. brassicae* from a library for which they found some homologies to fungal genes.

Changes in the host upon infection with *P. brassicae*

In a compatible reaction, signs of induced defence in the host root hair have been reported as deposition of callose between the host plasma membrane and the cell wall at the penetration site of the pathogen (Aist and Williams 1971). During the second infection cycle no evidence for an induced defence reaction was found. Williams and McNabola (1967) pointed out that necrotic responses were never observed and they discussed this as a reflection of the high degree of compatibility between host and parasite. Based on the observation of an enlarged host nucleolus, they noticed that the maintenance of a meristematic condition appears to be the chief response of the host cell to the parasite. Also, they discussed that the integrity of the outer membrane of the plasmodial envelope, which surrounds the plasmodium, may be under host control since its degeneration occurs at the same time as host ribosomes break down.

The most damaging aspect for the host in the infection course is the pronounced cell enlargement and cell proliferation. These symptoms, as well as the growth of leaf-like teratomata from the roots, indicate the involvement of the plant hormones auxin and cytokinin in disease development. At the same time, the gall is established as a sink tissue for photosynthetic products. Some of the results discussed below are summarized in a model in Fig. 3.

The availability of hormone-responsive reporter genes in *Arabidopsis* enabled the localization of enhanced auxin and cytokinin responsiveness to the part of the root where gall formation occurs (Siemens et al. 2006). Experiments by Dekhuijzen (1976) indicated that infected callus tissue was not hormone autonomous. There is evidence that the pathogen is able to synthesize cytokinins (Müller and Hilgenberg 1986), but for auxins this has not yet been demonstrated (Ludwig-Müller 1999).

With the introduction of *Arabidopsis* to clubroot research it was possible to perform genome-wide transcriptome analysis to investigate host gene expression during the development of the disease on a broader basis (Siemens et al. 2006). Two time points were chosen, which were significantly different from each other. At an early time point (10 days after inoculation) small secondary plasmodia of the pathogen were visible, but only about 20% of the host tissue was colonized with limited change of host cell and root morphology. At a later time point (23 days after inoculation) different developmental stages of the pathogen were present. More than 60% of the host root cells were colonized and the root morphology was drastically altered. This experimental setup will be referred to as ‘early time point’ and ‘late time point’ throughout the text. Similarly, Devos et al. (2006) carried out a proteome analysis but during even earlier stages after inoculation (4 days). Therefore the data set is not comparable to the transcriptome data, but provides additional information about changes in the root caused by *P. brassicae*.

Auxins and indole glucosinolates

So far it has to be assumed that the increase in indole-3-acetic acid (IAA) is derived from the host plant. The biosynthesis of IAA in Brassicaceae involves several possible pathways which are interconnected and might occur simultaneously. Which pathway is operating can be dependent on the developmental stage, or stress situation of a given plant species or a certain tissue

within a plant (for a recent review on auxin biosynthesis and metabolism see Woodward and Bartel 2005). Since indole glucosinolates are connected to auxin biosynthesis in Brassicaceae, we will concentrate on the changes in auxin and indole glucosinolate metabolism connected to gall formation in two different species, *Arabidopsis* and *Brassica*. Already early work on clubroot showed that during clubroot formation, an increased synthesis and turnover of the putative host auxin precursors (see Fig. 2) indole-3-acetaldoxime (IAOx), indole-3-methylglucosinolate (indole GSL) and indole-3-acetonitrile (IAN) have been detected in infected *Brassica rapa* roots (Searle et al. 1982; Rausch et al. 1983; Butcher et al. 1984). Based on this experimental evidence it was hypothesized that if the indole GSL are key factors for the biosynthesis of IAA in clubs, then plants having little or no indole glucosinolates, and as a result less auxin, might be resistant to clubroot or should develop less severe symptoms (Butcher et al. 1974). However, conflicting results on this topic have been presented in *Brassica* species, not allowing any definite conclusion (Butcher et al. 1974; Ockendon and Buczacki 1979; Chong et al. 1981, 1984; Mullin et al. 1980; Ludwig-Müller et al. 1997; summarized in Ludwig-Müller 1999). On the other hand, the glucosinolate content of non-*Brassica* plants including Caricaceae, Resedaceae and Tropaeolaceae, which were inoculated with *P. brassicae*, also increased compared to control plants and small galls were occasionally detected in *Tropaeolum majus* (Ludwig-Müller et al. 1999b) and *Lepidium sativum* (Butcher et al. 1976). It was therefore speculated that these GSL (mainly benzyl GSL) could serve as precursors for phenylacetic acid, a naturally occurring auxin in *T. majus* (Ludwig-Müller and Cohen 2002).

With the availability of transgenic plants or mutants it was possible to further test this hypothesis. A set of mutants which were selected for changes in glucosinolate patterns (Haughn et al. 1991) was used to demonstrate that *tu8*, reduced in leaf indole GSL, showed reduced club fresh weight (Ludwig-Müller et al. 1999a), which correlated with lower IAN and lower free IAA content in the mutant roots compared to the wild-type, and the development of the pathogen seemed retarded. The *TU8* gene encodes the *Arabidopsis* heterochromatin-like protein1 (Kim et al. 2004), and therefore a direct connection to indole GSL could not be demonstrated.

A double mutant in the cytochrome P450 enzymes CYP79B2 and CYP79B3, which are involved in the first step of the biosynthesis of indole glucosinolates (Hull et al. 2000; Mikkelsen et al. 2000) and are indole glucosinolate-deficient, showed no differences in gall formation compared to wild-type plants (Siemens et al. 2007), although free IAA levels were comparable to the wild-type in the mutant galls. This demonstrates that indole glucosinolates are not the primary source of elevated levels of IAA in galls of mutant plants. One explanation might be that a block early in the pathway (Fig. 2) can compensate IAA levels possibly via a different route, whereas blocking steps later in the pathway, i.e. nitrilase (see below), does not allow for alternatives, thus resulting in smaller galls with less IAA.

The myrosinase-glucosinolate system present in crucifers is involved in several aspects of plant development and defence. Hydrolysis of GSL by myrosinase enzymes can produce substances with a remarkably wide spectrum of biological activities in addition to precursors for plant hormones (Halkier and Gershenzon 2006). One route for indole GSL metabolism is the conversion by myrosinase to IAN (Fig. 2). Myrosinase transcript was induced during late stages of clubroot in *B. rapa* (Grsic et al. 1999) and Devos et al. (2006) found an induction of a myrosinase in *Arabidopsis* by proteome analysis during very early stages of infection.

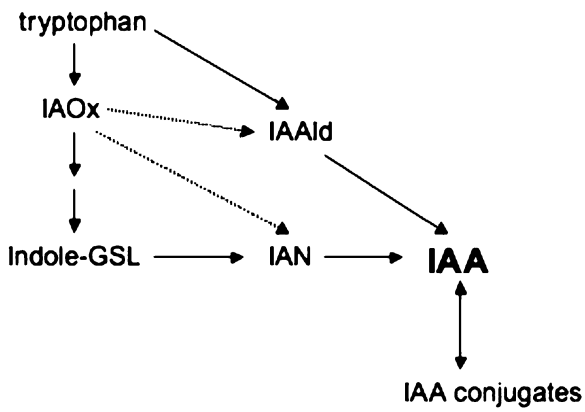

Fig. 2 The major pathways to indole glucosinolate and IAA synthesis possibly regulated during clubroot. *Solid arrows* indicate that for these pathways genes are known which encode the enzymes necessary for conversion, *dashed arrows* indicate that this pathway has been postulated or that enzymatic activities have been found, but so far no genetic evidence exists for the pathway

The next step in the IAA biosynthetic pathway would be the conversion of IAN to IAA by nitrilase (Fig. 2). Not surprisingly, nitrilase mRNA was increased during late stages of gall formation in *B. rapa* accompanied by an increase in nitrilase activity (Grsic et al. 1999). Transcript induction for nitrilase in *Arabidopsis* also correlated with the nitrilase protein localized exclusively in cells containing sporulating plasmodia (Grsic-Rausch et al. 2000; Neuhaus et al. 2000), indicating a role for this enzyme in cell enlargement (Grsic-Rausch et al. 2000). The *Arabidopsis nit1* mutant was more tolerant to clubroot, and antisense plants for the *NIT2* gene revealed retarded gall development (Grsic-Rausch et al. 2000; Neuhaus et al. 2000). In *B. rapa*, a different pathway for IAA synthesis via the intermediate indole-3-acetaldehyde (IAAld) might be also operating, because aldehyde oxidases were up-regulated during clubroot formation (Ando et al. 2006a). However, from transcriptome analysis there is no evidence that in *Arabidopsis* the corresponding genes are differentially expressed.

Since the nitrilase pathway seems to be induced only during later stages of infection, another possibility for the increase of IAA during earlier time points has to be considered. An additional pathway to control free IAA levels is the hydrolysis from inactive conjugates (Fig. 2; Woodward and Bartel 2005). Ludwig-Müller et al. (1996) have described and characterized an auxin conjugate hydrolase activity from *B. rapa* which showed an increased enzyme activity in *P. brassicae*-infected roots compared to controls. In addition, four different amidohydrolase genes with high homology to the respective *Arabidopsis* genes were cloned from infected *B. rapa* roots (Schuller and Ludwig-Müller 2002, 2006). Using Real Time RT-PCR it was not possible to conclusively demonstrate the induction of hydrolase mRNA during infection (Schuller and Ludwig-Müller 2006). This result, together with the observation that the *B. rapa* hydrolases prefer IAA-alanine as substrate, led to the hypothesis that the activity found in root galls specific for IAA-aspartate, a conjugate most likely involved in the degradation of IAA but not in its hydrolysis (Östin et al. 1998), is not a substrate for the host hydrolase family. Therefore, maybe *P. brassicae* could be involved in IAA conjugate hydrolysis (Fig. 3), but this hypothesis has yet to be tested. The strong increase of IAA conjugates in *B. rapa* at different time points during infection (Ludwig-Müller et al. 1996; Devos et al. 2005) may also be indicative of increased IAA synthesis and subsequent conjugation. The latter may avoid a potentially toxic accumulation of IAA in the infected plant (Devos et al. 2005). Although transcriptome analysis did not show evidence for the up-regulation of auxin conjugate hydrolases in *Arabidopsis*, a proteome approach revealed IAR4, identified in a screen for

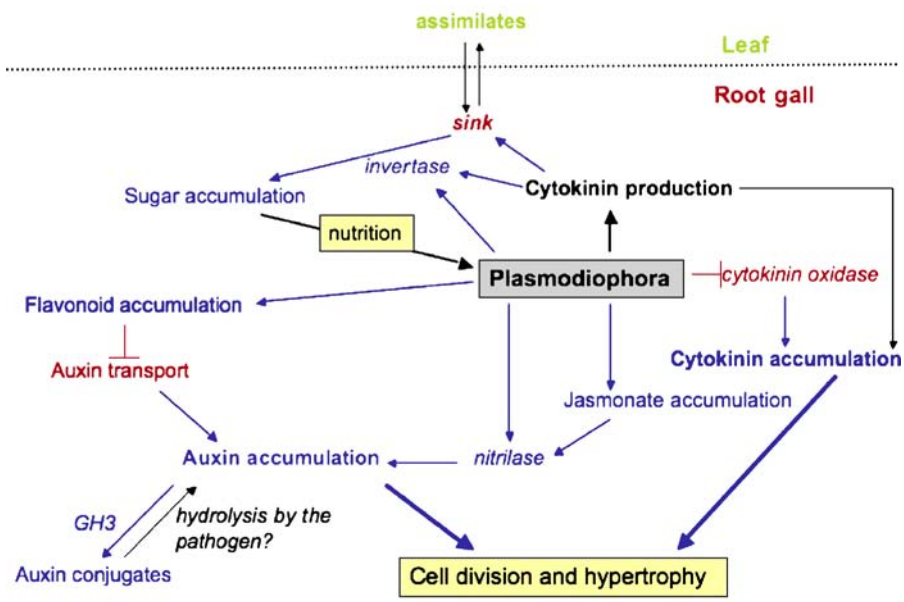

Fig. 3 A current model explaining the findings on hormone homeostasis and metabolism in clubroots of *Arabidopsis thaliana*. *Red* denotes down-regulation, *blue* up-regulation of pathways, compounds, enzymes or genes. *Black arrows* indicate that the pathway is directly influenced by *P. brassicae*. Data are summarized from direct experimental evidence or transcriptome analysis

auxin conjugate resistance, as an upregulated protein (Devos et al. 2006). The enzyme possibly acts on the conversion of indole-3-pyruvate to indole-3-acetyl-coenzyme A, which is a potential precursor of IAA amino-conjugates (LeClere et al. 2004). The reverse reaction from free IAA to auxin conjugates is catalyzed by six members of a large gene family in *Arabidopsis*, called *GH3*, which adenylate IAA and form amino acid conjugates (Staswick et al. 2005). Several *GH3* genes are auxin-inducible which makes them ideal tools to control IAA levels when excess auxin is present. The strong up-regulation of some members of the auxin-inducible *GH3* family involved in IAA amino acid conjugate formation point to the crucial role of auxin homeostasis during club development (Horn et al. 2006; Siemens et al. 2006). It is interesting to note that one conjugate formed is with aspartate (Staswick et al. 2005), linking IAA conjugate formation to the possibility that IAA amino acid conjugates with aspartate could be hydrolyzed by the pathogen (Fig. 3).

Also, several auxin-induced genes and auxin response factors were differentially regulated. The *axr3* mutant coding for the Aux/IAA protein IAA17 (for a recent review of the function of these proteins see Quint and Gray 2006), was more tolerant to clubroot (Alix et al. 2007). Other mutants connected to auxin metabolism or signalling were not affected (e.g. *axr1*, *axr2*, *ilr1*; Siemens et al. 2002). Furthermore, several putative auxin transport proteins were up-regulated according to transcriptome analysis, although a mutant of the auxin influx carrier AUX1 (Yang et al. 2006) did not show any differences in gall formation compared to the wild-type (Siemens et al. 2002). Differences in the observation that some mutants did not show tolerance or resistance even though their transcripts were increased, might be due to their position and importance in the pathway, whether the mutation can be compensated by others and whether parallel pathways are present. Since the IAA distribution seems to be important for gall development (Devos et al. 2006) it is tempting to speculate that fine tuning of the auxin transport pathway is also involved in regulating the auxin response (Fig. 3). Flavonoids are discussed as modulators of auxin efflux carriers of the PIN family (Besseau et al. 2007; Peer et al. 2004) and the accumulation of these compounds has been observed in root galls (Ludwig-Müller et al. 2006).

Cytokinins

It was shown that the amount of free and bound cytokinins was two to three times higher in clubs than in control roots (Dekhuijzen 1980). Dekhuijzen (1981) also showed that the contents of bound and free cytokinins were different in the host cytoplasm and plasmodia of the pathogen. Plasmodia contained zeatin riboside and its glucose derivative, whereas the host cytoplasm contained zeatin riboside and small amounts of the glucose-6-phosphate derivatives of zeatin and zeatin riboside (Dekhuijzen, 1981). From these results, it was concluded that plasmodia synthesize cytokinins which are released into the host cytoplasm inducing host cell division. Müller and Hilgenberg (1986) isolated young secondary plasmodia from clubs 23 days after inoculation by a two-step percoll gradient and showed that isolated secondary plasmodia of *P. brassicae* were not only able to take up ^{14}C-adenine in vitro, but also to incorporate adenine into *trans*-zeatin. It can thus be assumed that the increase in cytokinins is at least partly derived from active synthesis of zeatin by plasmodia of *P. brassicae*. The authors speculated that only a very small amount of *trans*-zeatin with its high biological activity would be sufficient to induce a sink in clubbed roots (Müller and Hilgenberg 1986). Transcriptome data showed that putative cytokinin biosynthesis genes as well as root-specific cytokinin oxidases/dehydrogenases, which are involved in the degradation of cytokinins (Werner et al. 2003), were down-regulated in *Arabidopsis* root galls. The down-regulation of the cytokinin-degradation capacity within colonized cells in combination with cytokinin production inside these cells by the pathogen can turn these cells into strongly dividing tissue by small amounts of pathogen-derived cytokinin (Siemens et al. 2006). This is strongly corroborated by the fact that lowering cytokinin levels by overexpressing a cytokinin oxidase gene in *Arabidopsis* leads to strong tolerance against *P. brassicae* infection (Siemens et al. 2006). However, in *B. rapa* in contrast to the situation in *Arabidopsis*, an increased transcription of isopentenyl transferase genes, involved in cytokinin biosynthesis, was described in root galls (Ando et al. 2005). These differences might be explained by differences in the host life-cycle because for *Arabidopsis*, only whole root systems were analyzed, whereas individual parts of the root were investigated in the study with *B. rapa*. The authors have separated gall tissue from tissue

without symptoms and also looked at younger or older parts of the gall which could influence the outcome of such an analysis (Ando et al. 2005).

Cytokinins are also known to be involved in the attraction of nutrients. The induction of a strong metabolic sink by *P. brassica* has been described decades ago (Keen and Williams 1969a, b). Reallocation of assimilates from leaves to infected hypocotyls has been detected in this phase of infection (Keen and Williams 1969b). Evans and Scholes (1995) showed the redirection of soluble sugars and the altering of carbon partitioning in infected *Arabidopsis* plants. Starch accumulation has been observed (Brodmann et al. 2002), but starch synthesis mutants (*adg1*, *adg2*) of *Arabidopsis* did not showed any degree of tolerance to clubroot infection (Siemens et al. 2002). Most of the genes involved in sugar or starch metabolism were up-regulated either at the first or second time point, including sucrose synthase, invertase, starch synthase, and ß-amylase (Siemens et al. 2006). In this context it is interesting to note that invertases can be induced by cytokinins (Ehneß and Roitsch 1997). In addition, several sugar transporters were differentially regulated, which indicates a strong change in trafficking of metabolites between different cells and compartments. Interestingly, a trehalase gene was also up-regulated at the late time point, which might be involved in the control of trehalose levels in the plant, because *P. brassicae* produced trehalose in its resting spores (Brodmann et al. 2002). The disaccharide trehalose is a storage form of carbon but also a protectant against various stresses such as desiccation and heat stress in bacteria and yeast (Müller et al. 1999). In an analogy, it could be seen as storage in clubroot-diseased *Arabidopsis* plants because trehalose was found only in infected tisues (Brodmann et al. 2002), but it is not clear whether the trehalose produced in plant tissue is derived from the pathogen. However, it could also protect the resting spores of *P. brassicae* from desiccation.

Hormones involved in biotic and abiotic stress signalling

Abscisic acid (ABA), ethylene, jasmonic acid (JA), and salicylic acid (SA) are plant hormones that play important roles during abiotic and biotic stress signalling. With respect to biotic stress, there is evidence that several signalling pathways involving plant hormones exist, but not all compounds are involved at the same time. It is a generally accepted view that, for example, ethylene acts synergistically with JA in the activation of defences against necrotrophic pathogens and as an antagonist in the SA-dependent resistance against biotrophic pathogens. However, SA and ethylene can also act together in the control of resistance to selected biotrophs. Consequently, mutants with constitutive ethylene, jasmonate or salicylate response are more tolerant to biotrophic pathogens (for a recent review on the complex networks see e.g. Adie et al. 2007). While there is increasing knowledge on signalling pathways during infection of *Arabidopsis* with leaf pathogens, no such evidence has been detailed for the root pathogen *P. brassicae*.

The regulatory function of abscisic acid has been extensively studied in relation to plant abiotic stress responses, such as drought, salt, and cold (Davies et al. 2005; Gusta et al. 2005), but it has recently been acknowledged to regulate responses to biotic stress as well in an antagonistic manner with ethylene and JA (Adie et al. 2007). First indications that ABA plays a role during clubroot have been obtained from *B. rapa* roots (Devos et al. 2005). These authors found an increase in ABA when gall development occurred. Analyzing the transcriptome data of *Arabidopsis* concerning the ABA influence during clubroot disease indicates that genes coding for ABA responsive/induced proteins as well as genes that are involved in ABA signalling were strongly increased during the late time point. In addition, several drought-responsive genes were also up-regulated (the total dataset of the microarray can be found at Array Express of the European Bioinformatics Institute, experiment no. E-MEXP-254).

For genes involved in ethylene synthesis or genes annotated to be ethylene-responsive, no consistent pattern was obtained, although several genes encoding biosynthetic enzymes and ethylene response factors were differentially regulated according to the microarray data. Mutant analysis showed that ethylene response mutants were not tolerant to clubroot (Siemens et al. 2002; Alix et al. 2007). However, the *alh1* mutant, defective in the cross-talk between ethylene and auxins (Vandenbussche et al. 2003), probably at the site of auxin transport, shows a resistant phenotype upon *P. brassicae* infection (Devos et al. 2006).

JA and its methyl ester have been found in a large number of plant species (Parthier 1991). They are known to regulate a number of different physiological processes in plants including the induction of senescence, vegetative storage proteins, and proteinase inhibitors (Koda 1992). They also have a role in interplant signalling (Pena-Cortes et al. 2004) and in signal transduction in relation to defence gene induction (Pauw and Memelink 2004; Pozo et al. 2004). Endogenous JA concentrations increased between 21 and 35 days after inoculation in infected *B. rapa* roots (Grsic et al. 1999) indicating a role for JA as a signal during clubroot development. Analysis of the transcription of genes involved in the jasmonate biosynthesis pathway showed a strong increase in expression for one lipoxygenase gene at the late time point, whereas genes encoding the following proteins in the biosynthesis pathway were either not regulated or they had rather a tendency to show down-regulation. The gene encoding JAR1, the enzyme responsible for adenylation of JA and subsequent synthesis of JA amino acid conjugates (Staswick et al. 2002; Staswick and Tiryaki 2004) was down-regulated and consequently the *jar1* mutant was more sensitive to clubroot (Siemens et al. 2002). It was also shown that indole GSL is selectively induced by jasmonic acid (JA) or methyl-JA treatment in *B. rapa* (Ludwig-Müller et al. 1997) and that nitrilase and myrosinase were inducible by JA (Grsic et al. 1999). These findings indicate possible links between auxin and JA response during clubroot development.

Conclusion

A general problem researchers have to deal with during biochemical and molecular investigations on clubroot is the intimate contact between pathogen and host. The data available so far have mostly been obtained with total root material so that very subtle changes of metabolism or gradients in hormone concentrations could not easily be detected when infected and healthy tissues were compared. One has to take into account that the pathogen may be able to induce local changes, which have to be resolved on a cytochemical level. Therefore, it will be necessary in the future to obtain data using either immunocytological studies, as has been done with antibodies against nitrilase (Grsic-Rausch et al. 2000), or with promoter-reporter lines which will allow at least detection of responsiveness to hormones on a more cellular level (Siemens et al. 2006). This approach will of course also allow the monitoring of other transcriptional activities in more detail. Also, the use of reverse genetics or tissue-specific expression of transcripts to change gene expression in a pathogen-inverse manner will allow us to investigate the function of individual genes in the context of clubroot disease.

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