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Genius Architect or Clever Thief—How *Plasmodiophora brassicae* Reprograms Host Development to Establish a Pathogen-Oriented Physiological Sink

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When plants are infected by *Plasmodiophora brassicae*, their developmental programs are subjected to extensive changes and the resultant clubroot disease is associated with formation of large galls on underground tissue. The pathogen's need to build an efficient feeding site as the disease progresses drives these changes, ensuring successful production of resting spores. This developmental reprogramming is an outcome of interactions between the pathogen and the infected host. During disease progression, we can observe alteration of growth regulator dynamics, patterns of cell proliferation and differentiation, increased cell expansion, and eventual cell wall degradation as well as the redirection of nutrients toward the pathogen. Recently, detailed studies of anatomical changes occurring during infection and studies profiling transcriptional responses have come together to provide a clearer understanding of the sequence of events and processes underlying clubroot disease. Additionally, genome sequencing projects have revealed *P. brassicae*'s potential for the production of signaling molecules and effectors as well as its requirements and capacities with respect to taking up host nutrients. Integration of these new findings together with physiological studies can significantly advance our understanding of how *P. brassicae* brings about reprogramming of host development. This article summarizes the current state of knowledge on cellular changes induced by *P. brassicae* infection and aims to explain their impact and importance for both the host and the pathogen.

Keywords: clubroot, gall development, gall-forming pathogen, microscopy and imaging, *Plasmodiophora brassicae*, protist–plant interactions

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Plasmodiophora brassicae is a biotrophic protist plant pathogen infecting, mainly, members of the Brassicaceae family (Ludwig-Müller et al. 1999). Its life cycle is governed by the soil and water environments it occupies. Resting spores can survive in the soil for many years, germinating only when optimal conditions for infection occur. The first stage of infection is by haploid zoospores encysting on root hairs of the host plant. Inside infected root hairs, multinucleate primary plasmodia form and develop into zoosporangia which release zoospores back into the soil to act as a source for secondary infection. Secondary zoospores invade the roots of plants and develop into motile secondary plasmodia which are responsible for further expansion and establishment of the disease within the plant (Kageyama and Asano 2009). Early stages of secondary infection are accompanied by massive changes in host metabolism which sets up a new, pathogen-oriented, physiological sink (Devos et al. 2006). This results in hyperplasia followed by hypertrophy of the underground parts of the plant and the development of galls (Malinowski et al. 2012). Macroscopically, this can be observed as a major root and hypocotyl deformation, hence the disease name: clubroot. The resulting structures secure space for pathogen multiplication and act as a perfectly tailored feeding site. The significance of galls as a specialized feeding site has been illustrated by detailed studies on *P. brassicae*-driven alterations of host sugar metabolism (Evans and Scholes 1995; Keen and Williams 1969) as well as anatomical changes facilitating acquisition of plant assimilate by the pathogen (Malinowski et al. 2012). Gall architecture changes during disease progression in response to pathogen needs. Initially, they support colonization of large numbers of cells and, subsequently, they act as a new, competing physiological sink fulfilling the nutritional requirements for spore maturation. Once spores mature, the disintegration of underground plant parts occurs and spores are released to the soil. As we see, completion of the entire life cycle of the pathogen is accompanied by complex rearrangements of the host. Here, we would like to present recent progress in understanding host cellular changes driven by *P. brassicae* infection and explain the ways in which particular features of gall architecture facilitate successful biotrophy.

MERISTEMATIC ACTIVITY CHANGES AND THEIR IMPLICATIONS FOR PLANT ANATOMY

Plants acquire their final form and maintain the functions of particular organs through the action of local sites of meristematic activity (Jürgens 2003). The apical-basal axis is formed through the action of the primary root and shoot meristems.

During later stages of plant development, as a consequence of local proliferative activity within the pericycle, lateral root meristems emerge. In the upper parts of plants, auxiliary meristems are formed and their action determines shoot branching. Radial secondary growth of plant organs is regulated by vascular cambium and phellogen activity coordinated by phytohormones. It is known that *P. brassicae* infection affects the levels of growth regulators which control meristematic activity (Ludwig-Müller et al. 2009; Schuller et al. 2014). Much effort has been directed toward dissecting the role of phytohormones in clubroot disease but a clear picture has yet to fully emerge. Recent works show that, depending on the genotype, different patterns of hormonal changes were found to correlate with the capacity of the host to trigger defense responses against *P. brassicae* infection (Lemarié et al. 2015; Prerostova et al. 2018). Downstream cellular changes triggered by *P. brassicae* in roots and hypocotyls seem to be more conserved. The first indications of changes in meristematic activity can be observed in the underground parts of plants as early as 4 days post-infection (dpi) as an increase in proliferation in the cortex of infected roots and hypocotyls (Devos et al. 2006). At this stage (18 days after germination), infection also enhanced local cell cycle activity within the pericycle, at the sites of lateral root meristems. The process of lateral root formation is, to a large extent, regulated by auxins (Casimiro et al. 2001), which are also known to mediate host responses to *P. brassicae* infection (Jahn et al. 2013). Plants respond to *P. brassicae* infection with increased levels of the TIR1 auxin receptor as well as Gretchen Hagen 3 (GH3) auxin conjugate synthases involved in controlling the homeostasis of auxin levels; the expression of downstream responses mediated by auxin response factors is also upregulated.

As the disease progresses, an intensification of existing meristematic activities in the vascular cambium occurs. In *Arabidopsis* clubroot gall development, a clear distinction can be made between the proliferative stage of gall formation related to cambial cell divisions and the later expansive phase, which involves organ hyperplasia and formation of enlarged cells (Fig. 1). Whereas in noninfected plants the progeny of cells in the cambial meristems advance toward differentiation, in *P. brassicae*-infected hypocotyls, they remain trapped in the mitotic state (Olszak et al. 2019). Suppression of cambial cell differentiation occurs at the late G2/M cell cycle progression checkpoint and is at least partially mediated by the dimerization partner DP, retinoblastoma RB-like, E2F, and MuvB (DREAM) regulatory complex (Olszak et al. 2019). In particular, transcript levels of *MYB3R1* and *MYB3R4* as well as *E2Fa* transcription factors, known to be responsible for activation of G2/M-specific genes, were elevated during the proliferative phase of gall development (7 to 18 dpi) in *Arabidopsis*. This was also accompanied by increased expression of DREAM targets, whose promoters contain the MSA motif such as B-type cyclin genes and cyclin-dependent kinases (Fig. 1). Once the production of resting spores starts, intense proliferation of host cambial cell progeny is no longer observed; instead, localized cell enlargement becomes the dominant pattern (Fig. 1). Thus far, it is not known which factors are directly involved in this switching of the root and hypocotyl cell fate programs. However, it has been shown that resting spore development is accompanied by host responses facilitating cell expansion, in particular those related to auxin, cytokinin, and brassinosteroid (BR) metabolism (Schuller et al. 2014). During this expansive stage of gall development, manipulation of host cell cycle control shifts to stimulate endoreduplication (Olszak et al. 2019). Endocycling accompanies organ growth and is essential for ploidy-driven cell size increase (De Veylder et al. 2011). For other biotrophic plant-microbe interactions, endoreduplication has been shown to be an important element facilitating the enhancement

of metabolic activity in host cells (Chandran et al. 2010). The importance of host endoreduplication in the formation of large, spore-filled cells likely rises from both its potentiation of increased cell volume to provide space for more spores and its impact on boosting cellular metabolism to provide nutrients for their multiplication and maturation.

The pathogen-driven modification of host meristematic activities also profoundly affects vascular tissue development, resulting in a dramatic repatterning. Both phloem and xylem development are affected; most likely, however, as a consequence of activation of two separate signaling cascades. Surprisingly though, *P. brassicae* does not influence the canonical CLE41/44-PXY-WOX4 cambium proliferation or differentiation regulatory pathway responsible for the balance between phloem and xylem development. Mutation of the *WOX4* gene does not limit *P. brassicae*-driven cambium proliferation and transcript levels of *PXY*, *CLE41*, *CLE44*, and *WOX4* genes are unaltered during the proliferative stage of gall formation (Malinowski et al. 2012). Expression patterns of the host genes regulating xylem differentiation observed during *P. brassicae* infection show that the xylogenesis process is already affected at an early signaling stage (Malinowski et al. 2012). In developing galls, the expression of two key transcription factors stimulating commitment of cell fate to xylogenesis decreases: *VND7* involved in the establishment of protoxylem and *VND6* involved in metaxylem differentiation (Malinowski et al. 2012). Downregulation of *MYB46* indicates that the machinery of secondary cell-wall synthesis necessary for xylogenesis is also suppressed. The final step of xylem formation, achieved through programmed cell death, is also restricted by downregulation of the cysteine protease genes *XCP1* and *XCP2*. It is possible that some clubroot-driven xylem restriction may be dictated by altered miRNA dynamics. It has been shown in cabbage that *P. brassicae* infection leads to an increase in miR156 abundance (Verma et al. 2014) and miR156 is an important element in the network regulating juvenile-to-adult phase transition, a process that is accompanied by hypocotyl xylem expansion (Ragni et al. 2011). Despite the fact that disruption of xylogenesis by *P. brassicae* has been widely reported and is a characteristic symptom of clubroot disease linked to plant wilting, we do not know if the signal triggering this response comes from the pathogen or is a consequence of host phytohormone homeostasis destabilization. Because *P. brassicae* infection also arrests xylogenesis in a quadruple cytokinin biosynthesis mutant *ipt1;3;5;7* incapable of accumulating cytokinin, the first hypothesis seems to be more plausible (Malinowski et al. 2016). Xylogenesis is under the control of networks regulating the balance between cell proliferation and endoreduplication (Taylor-Teeples et al. 2015); disruption of this balance in *myb3r4* mutants and *E2Fa*-silenced plants did not rescue the xylogenesis process (Olszak et al. 2019). The release of multiple genome sequences for *P. brassicae* in recent years has stimulated the hunt for pathogen effector proteins responsible for manipulating host processes (Pérez-López et al. 2018). The identification of effectors responsible for directly influencing xylogenesis would be of great interest because such a discovery could lead to strategies for mitigating the water distribution problems arising from infection which have such a critical impact on crop yield.

The altered balance between proliferation and differentiation observed in hypocotyls of *P. brassicae*-infected plants also influences the meristematic activity within phloem bundles. Altered phloem anatomy was first reported by Gustafsson et al. (1986), who pointed out that sieve cells dominate over tracheids in developing galls. Further characterization of phloem tissue in *P. brassicae*-infected hypocotyls, using the phloem-specific marker *CLE44* fused to the β -glucuronidase (*GUS*) reporter

gene, showed that disease does not change the number of phloem bundles but increases their cellular complexity (Malinowski et al. 2012). More detailed observations performed using electron microscopy showed that the number of all phloem cell types (companion cells [CC], phloem parenchyma, and sieve elements [SE]) within the bundle increased proportionally (Walerowski et al. 2018). This is consistent with the increased expression of numerous genes involved in early steps of phloem differentiation that accompanies gall development (Walerowski et al. 2018). Some of these critical regulators such as *BRX*, *CVP2*, and *OPS* act early in phloem fate acquisition and the very earliest steps of SE formation. Analysis of *ops-2* and *brx-2* loss-of-function mutants and the *cvp2-1 cvl1-1* double mutant which exhibit different degrees of phloem anatomy disruption has confirmed the involvement of these genes in the pathogen-driven reprogramming of phloem bundle complexity within galls. Infected plants carrying mutations in these phloem differentiation genes are much more severely affected by *P. brassicae* infection and die significantly earlier than wild-type plants. The life cycle of *P. brassicae* is accelerated in these mutants, with earlier formation of resting spores. Knowing that phloem can act as a conduit for both signaling and nutritional compounds, we can assume that this vascular reprogramming is a crucial element in fine tuning the biotic interaction between the host and pathogen. Because *P. brassicae* infection leads to increases in proliferation of phloem lineage cells, including phloem CC that are known to be involved in sugar loading and unloading (Slewinski et al. 2013), we propose that the importance of these changes to host vascular architecture will be to increase the availability of the host soluble sugars and that this availability will govern *P. brassicae*'s switch from multiplication to maturation.

THE GALL AS A SITE OF INTENSE CARBOHYDRATE CONSUMPTION

Sequencing of *P. brassicae* has revealed that, to a large extent, the pathogen relies on external sources of carbohydrates (Rolfe et al. 2016; Schwelm et al. 2015). To fulfill its

requirements, *P. brassicae* triggers redirection of assimilate in infected host plants. In $^{14}\text{CO}_2$ pulse-feeding experiments, Keen and Williams (1969) found that increased levels of highly radioactive sucrose but not hexoses were present in clubroot galls. Sucrose translocation stimulated by clubroot disease was also reported by Evans and Scholes (1995), who found that sucrose accumulation in leaves decreases upon *P. brassicae* infection. Both studies show that sucrose is the major sugar translocated from source to sink tissues as the disease progresses. Assimilate redirection toward developing galls can be achieved either at source sites via changes in soluble-sugar conversion and phloem loading or at the level of the sink by increased unloading. Also, phloem transport load can be stimulated. In *Arabidopsis*, sucrose is mainly loaded via active transport from the apoplast. Thus far, no studies describing the exact molecular mechanism of sugar loading in the source organs of *P. brassicae*-infected plants have been published. Irani et al. (2018) reported decreased levels of transcripts involved in photosynthesis as well as carbohydrate transporters in the upper parts of *P. brassicae*-infected plants. At the same time, root systems responded with upregulation of the Calvin cycle, tri-carboxylic acid (TCA) cycle, and genes implicated in carbohydrate transport or unloading. The unbalance between responses observed in source and sink tissues suggests that the host may attempt to limit assimilate hijacking by the pathogen by constricting the source. Limiting of sugar availability by decreasing transcription of genes involved in the light phase of photosynthesis and Calvin cycle in *Arabidopsis* leaves was also observed by Jubault et al. (2013). Concomitantly, starch biosynthesis genes were repressed, whereas those involved in starch degradation were induced. Despite the fact that infected plants limit their primary metabolism in source organs, the pathogen finds ways to relocate carbohydrates toward the sink they create as their feeding site. Increased metabolism of carbohydrates in the sink organs after infection was widely reported (Evans and Scholes 1995; Siemens et al. 2011). *P. brassicae* establishes a very strong carbohydrate sink by local stimulation of host extracellular cell wall invertases (cwINV) (Roitsch and Ehneß 2000). Recently completed

Fig. 1. *Plasmodiophora brassicae* infection changes meristematic cell fate by manipulation of the G2/M checkpoint. As a consequence of infection, existing host meristematic activity is maintained via the activation of dimerization partner DP, retinoblastoma RB-like, E2F, and MuvB (DREAM) complex members *MYB3R1*, *MYB3R4*, *E2Fa*, and their downstream targets, including B-type cyclins and cyclin-dependent kinases. Increased accumulation of these factors marks the proliferative phase of gall formation, in which stimulated cambial activity contributes to root and hypocotyl hyperplasia. As the disease progresses, unknown factors redirect cell fate at the G2/M checkpoint toward cell cycle exit and the endoreduplication process; this contributes to cell enlargement and the organ hypertrophy observed during the expansive phase of gall development.

P. brassicae genome sequencing shows that the pathogen itself does not possess extracellular invertase genes (Rolfe et al. 2016; Schwelm et al. 2015). In this light, we can speculate that the pathogen uptakes sugars in the forms delivered by the host. Upregulation of host sucrose invertases seems to be the most likely way for the availability of host sugar to be increased. Disruption of cwINV activity in galls permitted increased plant tolerance to *P. brassicae* (Siemens et al. 2011). A possible role of cwINV is to facilitate the uptake of apoplastic sucrose unloaded from the phloem tissue by metabolizing it to hexose. Roitsch and Ehneß (2000) as well as Walters and McRoberts (2006) suggested that cwINV activity can be an essential component of cytokinin-dependent carbohydrate sink formation. One possible scenario is that local increases in cytokinin levels induce extracellular invertase activity, influencing in this way sugar unloading and partitioning within the plant. If this is the case for clubroot disease, then increases in cytokinin levels should accompany the later stages of disease development. However, recent work by Malinowski et al. (2016) shows that the main forms of cytokinin do not increase during that time. We cannot exclude the possibility that *P. brassicae* itself can synthesize sufficient cytokinin to trigger extracellular invertase activity. In fact, at least two genes encoding isopentenyl transferases have been identified in the *P. brassicae* genome (Rolfe et al. 2016). In this light, it may be that pathogen-derived cytokinin can trigger assimilate redirection. It is notable, however, that *P. brassicae* spore development is delayed or even arrested in infected quadruple *ipt1;3;5;7* mutants (Malinowski et al. 2016). This suggests that the pathogen-derived pool of cytokinin is probably not sufficient to maintain proper sugar distribution for the pathogen's needs.

Competition between ordinary host sink organs and the pathogen-induced gall is further enhanced by the manipulation of host sugar transporter expression. Carbohydrates redirected by the pathogen are unloaded through the sucrose transporter (SUT) and sugars will eventually be exported transporter (SWEET) proteins. Expression of the host genes encoding these proteins is activated by the pathogen during the secondary phase of the infection, when cell enlargement follows cell proliferation (Malinowski et al. 2012; Walerowski et al. 2018). The upregulation of *SUC2/SUC1* and *SWEET11/12* is focused around the areas of phloem expansion in the gall, suggesting their involvement in unloading carbohydrate for the pathogen. Increased expression of *SUC2/SUT1* was observed at 16 and 26 dpi. However, further localization with promoter fusions showed similar patterns between hypocotyls of infected and noninfected plants at 16 dpi whereas, at 26 dpi, *SUC2/SUT1* signals disappeared in healthy hypocotyls (P. Walerowski and R. Malinowski, unpublished data). This suggests a possible connection of transporter expression with the late stages of gall development when there is a maximum carbohydrate requirement for resting spore formation (Fig. 2). Expression of two other sugar transporters, *SWEET11* and *-12*, was found to increase in hypocotyls of infected plants at 16 and 26 dpi. Consistent with this observation, mutations in *SWEET11* and *-12*, singly (Li et al. 2018) or in combination (Walerowski et al. 2018), slowed disease progression. In addition, Fourier-transform infrared microspectroscopy imaging has revealed the differences in carbohydrate distribution between galls of wild-type *Arabidopsis* and *sweet11;12* double mutants (Walerowski et al. 2018). Other host transporters possibly involved in increasing the supply of carbohydrates to *P. brassicae* are monosaccharide/H⁺ symporters of the sugar transporter protein family. Their increased abundance in galls was observed in *Arabidopsis* (Walerowski et al. 2018) and *Brassica oleracea* (Zhang et al. 2019); however, these observations have not yet been elaborated with functional studies. *P. brassicae*

also carries its own factors involved in sugar uptake that were found to be differentially expressed over the course of disease progression (Rolfe et al. 2016). Its genome contains SWEET permeases and monosaccharide transporters as well as other transporters that belong to unique classes. It also has endogenous mechanisms for carbohydrate metabolism (glycolysis and TCA cycle); however, better understanding of the involvement of these factors in particular steps of carbohydrate acquisition will require further functional analysis.

Another compound involved in reorientation of the carbohydrate sink during clubroot disease progression is trehalose. Brodmann et al. (2002) reported that plants infected by *P. brassicae* accumulate large amounts of this sugar. They also found that trehalose may, in fact, be synthesized by trehalose-6-phosphate synthase encoded by the *P. brassicae* gene *PbTPS1*. Trehalose-6-phosphate, which is an intermediate molecule of the trehalose synthesis pathway, regulates sucrose production in source organs and sucrose consumption in sink organs. It has also been shown to be involved in sucrose mobilization from starch during the night (Figueroa and Lunn 2016). Utilization of host substrates for trehalose synthesis could be an important mechanism facilitating the formation of a carbohydrate sink in developing galls. Apparently, infected plants try to cope with this metabolic destabilization either by induction of trehalase activity (Brodmann et al. 2002) or by adapting to tolerate high levels of this metabolite (Gravot et al. 2011). During late stages of gall development, when resting spores are formed, pathogen manipulation of carbohydrate metabolism may be important for maintenance of cell osmotic properties as well as for conversion of soluble sugars to starch when their levels become toxic.

CELLULAR ENVIRONMENT AND CELL INTEGRITY WITHIN THE GALL

As the disease progresses, cellular integrity in the gall is subjected to counteracting trends toward cell-wall reinforcement and cell-wall loosening as the developmental programs and needs of the host are subverted by the pathogen (Fig. 3). Gall development is also accompanied by cell-wall lesions or cell adhesion changes. In order to maintain its physiological functions, the host needs to counteract these changes. The cell wall is an essential cell component in determining cell fate; its physical and chemical properties influence cell expansion and the direction of cell growth as well as several aspects of differentiation into functional tissues (Thompson 2005; Wojtaszek 2000). Cell walls can be divided into those of primary and secondary type. Primary walls consist mainly of cellulose, hemicellulose, and pectins; they need to withstand mechanical force from turgor pressure and, thus, both their rigidity and extensibility require precise regulation. Secondary cell walls are additionally enriched with lignins and their synthesis usually occurs during cell growth arrest and while mechanical support or a physical barrier is needed. Schuller et al. (2014) showed that, to large extent, cell enlargement within developing galls is regulated by BR. Hypertrophied cell formation in galls is accompanied by increased transcription of genes regulating the BR biosynthesis pathway or encoding positive regulators of BR signaling. Their work also showed that BR mutants exhibit at least partially impaired resting spore formation or maturation. One possible scenario is that the pathogen hijacks the BR signaling pathway to modify the activity or abundance of cell-wall-loosening factors such as expansins (EXP) or xyloglucan endotransglucosylase hydrolases (XTH). It has also been shown that an increase in the xyloglucan endotransglycosylase activity of XTH proteins accompanies the early stages of *P. brassicae* infection (Devos et al. 2006) and may be related to an increase in epidermal root cell elongation processes observed

after *P. brassicae* infection. Other reports point out the primary cell wall extensibility modification by EXP as an important mechanism necessary for plant invasion by this pathogen and for subsequent gall formation (Jubault et al. 2013). BR may also regulate cell integrity within galls via the activation of pectin methylesterases (PME). Wolf et al. (2012) showed that a mutation in the *BR11* gene encoding the BR receptor can influence the increase of pectin esterification caused by PME gene overexpression. Increased PME levels were observed during the early stages of *P. brassicae* infection (Devos et al. 2006); this hyperesterification of pectins may lead to a reduction in cell integrity. Roots infected by *P. brassicae* are fragile and break apart easily

Another important aspect of the cellular environment that merits attention concerns the strategies that the pathogen has developed to cope with and subvert host defense responses. An important issue during establishment of the feeding site is the suppression of host immune mechanisms. At present, intense work aimed at characterization of potential *P. brassicae*-secreted molecules involved in modification of host responses is being carried out (Pérez-López et al. 2018). Thus far, the most well-characterized factor is *PbBSMT*, a secreted methyl transferase whose activity is related to suppression of salicylic acid (SA)-related defense signaling (Ludwig-Müller et al. 2015). Recently, using a chemically inducible system Bulman et al. (2019) has shown that increased *PbBSMT* levels correlated with higher clubroot susceptibility in the host. The authors propose that *P. brassicae* methylates SA, decreasing pools of free SA involved in host defense signaling. Other such mechanisms deployed by *P. brassicae* to create a favorable environment for gall formation and multiplication remain to be discovered.

In addition to evading and suppressing host defense responses, the pathogen within the developing gall will seek to manipulate metabolic pathways within occupied host cells. A consequence of the pathogen's success in establishing a strong physiological sink is the influx of metabolites, which must be marshalled for optimal spore formation. Recently completed sequencing projects have shown how the *P. brassicae* genome

has evolved to cope with this situation (Rolfe et al. 2016; Schwelm et al. 2015); genes encoding factors involved in carbohydrate and lipid metabolism or transport have been identified and their potential roles proposed. One key aspect emerging from these genome analyses is that *P. brassicae* has limited capacity for direct modulation of nitrogen uptake and metabolism. There is a possibility that the pathogen trehalose-6-phosphate synthase gene (*PbTPS1*), in addition to its role in sugar deposition, may also be important for the proper orchestration of carbohydrate and nitrogen levels in the host. Such a scenario has previously been observed in the rice blast fungus *Magnaporthe oryzae* (Fernandez and Wilson 2011).

The metabolic sink induced by *P. brassicae* is so strong that accumulation of certain molecules can become toxic; therefore, efficient mechanisms for cellular compartmentation or sequestration have to be deployed. Toxic levels of carbohydrates accumulating within gall cells are sequestered as starch granules; the pathogen can also act to neutralize toxic build ups of fatty acids (Fig. 3A). Bi et al. (2016) have shown that fatty acids are neutralized via esterification and subsequent sequestration in the form of neutral lipids (mainly arachidonic acid) in so-called “lipid droplets”. Similarly to Rolfe et al. (2016), they observed that synthesis of fatty acids in plasmodia is accompanied by increased expression of several *P. brassicae* lipase-encoding genes, and subsequent resting spore formation

Fig. 3. Serious cellular rearrangements within the gall during late stages of clubroot disease. **A**, Scanning electron microscopy (SEM) showing the characteristic phenotype of an enlarged cell colonized by the pathogen. Stars represent the following: green = host cell nuclei, magenta = starch grains accumulating in response to high levels of carbohydrates, and orange = *Plasmodiophora brassicae* plasmodium containing lipid droplets (marked with yellow stars). **B**, Typical cell-wall deformations of host cells within galls (26 days postinfection [dpi]) observed with SEM. **C** and **D**, Toluidine-blue-stained, Technovit-embedded sections of a gall at 32 dpi showing cell wall decay (indicated with yellow arrowheads) and lignification (turquoise cells marked with red arrowheads). Scale bars represent 10 μ m (**A** and **B**) and 100 μ m (**C** and **D**).

coincides with expression of numerous factors involved in lipid metabolism. Integrative analysis of isolated lipid droplets present at different stages of *P. brassicae* development have shown that their role goes far beyond the maintenance of proper cellular environment. These particles can also act as reservoirs that can be accessed when there is a critical cellular requirement for energy, cell membrane synthesis, or production of signaling molecules (Bi et al. 2016).

Metabolic and cellular changes within the developing gall are also tightly connected to auxins, which have been shown to accumulate in large quantities during late stages of disease, facilitating nutrient availability for developing resting spores (Ludwig-Müller 1993). It is known that *P. brassicae* can influence auxin metabolism by induction of host nitrilase genes, which modulate levels of free indole-3-acetic acid (Grsic-Rausch et al. 2000). Schwelm et al. (2015) reported that the *P. brassicae* genome contains a *PbGH3* gene that can regulate auxin homeostasis. The involvement of GH3 factors in developmental reprogramming may be part of a conserved signaling network exploited by pathogens for feeding-site formation. It has been shown that GH3 accumulation accompanies the early phases of symbiotic interactions between *Sinorhizobium meliloti* and *Medicago truncatula* (Yang et al. 2015). Because elevated host GH3 transcript levels were observed in hypertrophied cells colonized by *P. brassicae* (Schuller et al. 2014), it could be that they also act to modify the pathogen-driven auxin increase necessary for cell wall expansion. Due to the fact that auxin is involved in every single aspect of plant life, it is very difficult to dissect how a particular change affects gall formation and function; thus, more studies integrating the interplay with other growth regulators (particularly cytokinins) need to be performed to make a proper appraisal.

CONCLUDING REMARKS

Important progress has been achieved in understanding the cellular changes leading to gall formation and how this structure fulfils the needs of the pathogen. *P. brassicae* stimulates mitotic activity in the meristematic regions of roots and hypocotyls to increase the number of cells available to be colonized. Detailed understanding of this reprogramming at the cellular and molecular levels may help to develop strategies to reduce the negative impacts of *P. brassicae* infection on crop yield. The gall is also engineered to be a major carbohydrate sink, competing with other host sink organs such as young leaves or developing seed. A future challenge will be to identify and characterize the pathogen factors stimulating the host machinery that is used to establish a new metabolic sink and to siphon off carbohydrates.

Despite various attempts aimed at characterization of the phytohormonal response to infection and how this is manipulated by the pathogen our understanding of this aspect is, as yet, incomplete. This is largely due to the fact that phytohormonal imbalances were frequently studied in isolation whereas more integrative studies, taking into account hormonal cross-talks, are required.

Another level of complexity that should be explored while studying nutrient redirection during clubroot disease is the possible involvement of pathogen-derived agents capable of influencing metabolism and transport. Apart from pathogen-driven host metabolic reprogramming, further characterization of *P. brassicae* secreted molecules capable of suppressing host defense machinery may also bring new useful information that helps us to understand the disease progression. In our opinion, future development of an efficient method to modify the *P. brassicae* genome as well as exploration of the genomic diversity between particular pathotypes may open up new

avenues for the functional studies of the biological basis of clubroot disease progression.

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