

Molecular actors of seed germination and haustoriogenesis in parasitic weeds

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Update

Parasitic plants *sensu stricto* are defined by their ability to tap nutrients required for their growth directly from other plants, by invading the stems or the roots of their hosts through specific structures called haustoria (Wicke and Naumann, 2018). First records mentioning parasitic plants date back to the third century BC. Nevertheless, it is only in the 1950s that intensive research in their physiology began, when *Striga asiatica* was accidentally introduced into the United States where it rapidly and intensely altered maize (*Zea mays*) crops, then in the 1970s and 1980s when many African countries suffered from severe famines (Heide-Jørgensen, 2008). Research on parasitic plant biology is today experiencing a new boom, as a handful of species are increasingly damaging crops worldwide.

The world apart of parasitic plants

There are ~4,750 parasitic plants species, all grouped within dicotyledonous Angiosperms (Nickrent, 2020) at the exception of *Parasitaxus usta* that belongs to the Gymnosperms group (Feild and Brodribb, 2005). Parasitic plants represent 292 genera, grouped in *c.* 20 families, distributed on all continents except Antarctica (Westwood et al., 2010; Nickrent, 2020). However, parasitic plants have been and still are

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- The conditioning period revealed extensive DNA demethylation required for parasitic weeds to germinate in response to host-exuded chemicals.
- The diversification and subsequent neofunctionalization of HTL/KAI2 proteins have provided parasitic weeds with hypersensitivity to host-exuded SLs.
- Both SLs and non-SLs induce similar molecular responses in parasitic weeds, analogous to nonparasitic plants.
- Transcriptomes underlying haustoriogenesis in different lineages of parasitic plants converged to a similar list of genes that regulate root development, carbohydrate-active enzymes (CAZymes) activity, and hormone homeostasis in nonparasitic plants.
- Vascularization of the haustorium depends on genes that regulate vascularization in nonparasitic plants

subjected to extensive taxonomic rearrangements. Most recent phylogenetic analyses indicate 12 independent acquisitions of a parasitic lifestyle (Bromham et al., 2013). Consequently, parasitic plants display very high morphological variability and extremely diverse parasite strategies.

Parasitic plants are distinguished according to their site of attachment to the host. Thus, 40% are stem parasites that invade the stem of their host(s) (e.g., mistletoes and dodders), while 60% are root parasites that penetrate the host root system (e.g., broomrapes and witchweeds; Westwood et al., 2010). A second dichotomy separates facultative from obligate parasitic plants according to their degree of dependency to a host. Facultative parasites (e.g., *Triphysaria*, *Phtheirospermum*, and *Rhinanthus* spp.) are chlorophyllous organisms able to complete their life cycle in the absence of a host. However, in natural environments, most facultative parasites are likely to engage in parasitism as solitarily growing parasites suffer from impaired growth and reduced reproductive fitness as described in laboratory experiments (Kabiri et al., 2017; Honaas et al., 2019). Inversely, obligate parasites cannot survive without a host during at least part of their lifecycle (Irving and Cameron, 2009). Finally, most researchers differentiate flowering parasitic plants on the basis of their photosynthetic capacity. Approximately 10% of all flowering parasitic plants are achlorophyllous organisms. These so-called holoparasites are thus necessarily obligate parasites, and must take up water and nutrients (mainly reduced carbon and nitrogen) required for their growth by connecting to the xylem and phloem of their host through haustorial structures. By contrast, hemiparasites, whether facultative or obligate, are chlorophyllous and thus retain photosynthetic ability. Obligate hemiparasites can limit their spoliation to mainly water and mineral compounds from the xylem of the host once they reach photosynthetic maturity.

The *Orobanchaceae* family is the largest family of parasitic plants (Heide-Jørgensen, 2008). *Orobanchaceae* species are root parasites, presumably arisen from a single gain of the ability to form haustoria (Schneeweiss, 2013). It comprises the full spectrum of trophic specialization, with a broad range of host preferences and specificities. Most importantly, it contains species that have turned into weeds causing 20%–100% of crop yield losses at the expense of several billion dollars a year (Parker, 2012; Rodenburg et al., 2016). Among these pests are holoparasites of the *Orobanche* and *Phelipanche* genera (broomrapes), which together affect a wide spectrum of hosts (e.g., legumes, sunflower, tomato, oilseed rape, etc.) in Europe, North Africa, and Asia (Parker, 2012). In addition, *Striga* species (witchweeds) are considered the major biotic constraint to cereal production in sub-Saharan agriculture, with over 50 million hectares infested annually (Ejeta, 2007).

Broomrapes and witchweeds have acquired numerous functional adaptations allowing them to compete with crops for resources. As compared to nonparasitic plants and nonweedy parasites, they have adapted to sense the presence of host roots in their close vicinity to trigger

seed germination and, subsequently, differentiation of early haustorial structures (Delavault et al., 2017). After successful establishment of a vascular continuum with the host, the parasite acts as a supernumerary and dominant sink whereby it withdraws water, nutrients, and hormones necessary for its own, at the expense of the host's development (Péron et al., 2017). As the reallocated resources accumulate, the parasite develops an external bulbous structure, the tubercle. This structure then differentiates into an underground stem, from which an inflorescence emerges above-ground within a few weeks.

Broomrapes and witchweeds sustainability is ensured by a very high reproduction rate. Indeed, each mature flowering plant produces up to hundreds of thousands of microscopic seeds easily spread from one location to another. Most of the current dreadful parasitic weeds thrive well in temperate areas and they have remarkably expanded in response to global warming (Phoenix and Press, 2005). Consequently, the rhizosphere of host plants is easily replenished in the span of only one generation, and parasitic weeds are expected to dramatically expand to new territories in the near future (Fernández-Aparicio et al., 2016b).

This review intends to describe recent progress in understanding how parasitic weeds sense surrounding hosts and highlights advancements in their genetics.

Seed dormancy and germination in root parasitic weeds

The successful establishment of obligate parasitic weeds of the *Orobanchaceae* family in agro-ecosystems partly relies on a particular seed physiology. As compared to most facultative hemiparasites and nonparasites (except *Orchidaceae* species), broomrapes and witchweeds produce small seeds with an average size of 200 μm (Joel, 2013). A single mature plant annually releases tens to hundreds of thousands of these dust-like seeds, which can remain dormant and viable in the soil for more than a decade (Yoder and Scholes, 2010; Joel, 2013). The progressive release from the dormant state occurs, as for the many nonparasitic species displaying a physiological dormancy, upon perception of stable and adequate environmental conditions (Bewley et al., 2013; Baskin and Baskin, 2014).

Decades of research on nonparasitic models have identified multiple dormancy-breaking signals, among which appropriate temperatures and water and oxygen availability are arguably universal prerequisites in the decision to maintain or release physiological dormancy. The same rules perfectly apply to obligate parasitic weeds, whose dormancy release takes the form of a “warm stratification.” Also referred to as the “conditioning period,” it indeed requires a moist environment that initiates seed imbibition, and lasts from few days to several weeks at 18°C–30°C in *in vitro* conditions (Matusova et al., 2004; Lechat et al., 2012; Brun et al., 2019). A recent study identified ~15,000 differentially expressed genes that correlate with the acquisition of a conditioned state in *Phelipanche aegyptiaca* (Bao et al., 2017).

The authors found that key genes encoding phosphofructokinase (PFK) and pyruvate kinase (PK) involved in the glycolysis pathway are downregulated in conditioned seeds compared with unconditioned seeds (Bao et al., 2017). In the closely related species *P. ramosa*, the adenylate energy charge reaches a maximum of 0.9 within a day of conditioning (Lechat et al., 2012). These results together suggest that most of the energy required for subsequent germination is produced through the glycolysis pathway early during the conditioning phase, which constitutes a major response to water uptake. In addition, Lechat et al. (2015) demonstrated through a pharmacological approach in *P. ramosa* that global DNA demethylation must occur for the conditioning to be achieved. Similar patterns are observed upon early imbibition and in germinating seeds of multiple nonparasitic species (Portis et al., 2004; Lu et al., 2006; Meng et al., 2012; Bouyer et al., 2017; Kawakatsu et al., 2017; Narsai et al., 2017).

The current lack of genetically tractable systems along with the relatively low amount of “omics” datasets for *Orobanchaceae* species still hinder our understanding of the molecular mechanisms at play during the conditioning period. For instance, which molecular actors contribute to such extensive DNA demethylation, and what are the targeted genomic regions? The current lines of evidence indicate that the conditioning period in parasitic weeds resembles the process of dormancy release of nonparasites to a reasonable extent, which suggests that similar genetic and epigenetic programs might regulate both developmental frameworks. Accordingly, the purpose of dormancy release in both cases is to allow seeds to germinate at the proper time and place, hence upon appropriate germination factors. Broomrapes and witchweeds are unique in their ability to sense the presence of potential host roots in their close vicinity, which ensures that conditioned seeds do not germinate unless they perceive chemicals exuded by surrounding potential host roots into the rhizosphere. The functional gain of such a recognition system is critical to their survival since it prevents microscopic seeds from wasting their limited resources in the attempt to reach a suitable host. The most characterized germination stimulants are strigolactones (SLs), a family of plant hormones that also act in planta throughout the development of the host (Waters et al., 2017). More than 25 SLs have been identified since the first discovery of strigol as a germination stimulant for *S. lutea* (Cook et al., 1966), and recent reviews exhaustively describe SL chemical diversity and activities on the germination of parasitic weeds (Al-Babili and Bouwmeester, 2015; Cavar et al., 2015; Brun et al., 2018). There is a clear correlation between the ability of the parasitic weed to germinate upon a certain SL structure and its occurrence in the parasite's host(s) exudates (Fernández-Aparicio et al., 2009; Fernández-Aparicio et al., 2011; Ueno et al., 2014), suggesting that host specificity and range are at least partly determined by this chemical interaction. In some instances, the use of low SL producers has proven successful in inhibiting the

germination of parasitic weeds (Gobena et al., 2017). However, it is not universally practicable as many of them also exhibit submicromolar sensitivities to chemicals whose structures deviate from canonical SLs (Brun et al., 2018). For example, dehydrocostus lactone (DCL) exuded by sunflower stimulates germination of the sunflower-specific parasite *Orobanche cumana*, but not of other incompatible *Phelipanche* or *Striga* species (Joel et al., 2011; Brun et al., 2019). Similarly, a specific strain of *P. ramosa* displays a high sensitivity to 2-phenylethyl isothiocyanate (2-PEITC) present in the rhizosphere of oilseed rape on which it has adapted exceptionally well in the span of a decade (Auger et al., 2012; Brun et al., 2019; Stojanova et al., 2019).

Recent studies conducted in *P. aegyptiaca* and *S. hermonthica* have revealed that perception of such diversity of SLs occurs through multiple α/β hydrolases of the HTL/KAI2 family (HYPOSENSITIVE TO LIGHT/KARRIKIN INSENSITIVE2), with each protein displaying its own affinity toward specific SLs (Conn et al., 2015; Toh et al., 2015; Zhang et al., 2020). Interestingly, weedy *Orobanchaceae* species contain a higher number of HTL/KAI2 genes that display a faster rate of molecular evolution compared with nonweeds and nonparasitic species (Conn et al., 2015). This has probably led to a neofunctionalization of HTL/KAI2 proteins, since HTL/KAI2 in nonparasitic species perceive karrikins (KARs) but not SLs (Flematti et al., 2004; Nelson et al., 2009). KARs share partial structural similarity with SLs, although they are produced through combustion of plant material. This family of compounds acts as smoke-derived signals to promote seed germination of numerous nonparasitic species after forest burning, thus favoring recolonization of devastated ecosystems (Nelson et al., 2012; Flematti et al., 2015). While SLs and KARs are different signals leading to seed germination of either parasitic weeds or nonparasitic plants, both share signaling analogies. Upon KAR perception in nonparasites, the HTL/KAI2 receptor binds the F-box protein MORE AXILLARY BRANCHES2 (MAX2), which supposedly ubiquitinates the transcriptional repressor SUPPRESSOR OF MAX2-1 (SMAX1) for proteasomal degradation (Nelson et al., 2011; Stanga et al., 2013). Interestingly, parasitic weeds also contain functional MAX2 proteins that can rescue the *Arabidopsis max2* mutant (Liu et al., 2014; Li et al., 2016). In addition, several studies indicate that KAR and SL signaling pathways similarly lead to altered abscisic acid (ABA) and gibberellins (GA) homeostasis. Notably, smoke-treated seeds of *Nicotiana attenuata* display lower ABA levels and higher GA levels (Schwachtje and Baldwin, 2004), which corroborates results obtained with *A. thaliana* and *Lactuca sativa* seeds treated with purified KARs (Nelson et al., 2009; Gupta et al., 2019). Similarly, exogenous application of the synthetic SL analog *rac*-GR24, which induces both SL and KAR signaling in nonparasitic plants (Scaffidi et al., 2014), promotes ABA decrease and GA increase in the obligate root parasitic plants *S. hermonthica*, *P. ramosa*, and *P. aegyptiaca* (Lechat et al., 2012; Toh et al., 2012; Bao et al., 2017). Such hormonal changes are consistently correlated with differential

regulation of genes encoding ABA and GA biosynthetic and catabolic enzymes (Nelson et al., 2009; Lechat et al., 2012; Toh et al., 2012; Bao et al., 2017; Brun et al., 2019). These findings together illustrate that HTL/KAI2 signaling, although activated by distinct chemical cues in parasitic weeds and nonparasitic species, similarly stimulates a decrease in the ABA/GA balance, as happens in response to other major environmental cues favoring seed germination over dormancy (Finch-Savage and Leubner-Metzger, 2006; Holdsworth et al., 2008; Finkelstein, 2013).

ABA catabolism seems especially key to germination of parasitic weeds. Indeed, *CYP707A* genes encoding ABA catabolic enzymes are consistently upregulated early upon stimulation in multiple species of broomrapes and witchweeds (Lechat et al., 2012; Brun et al., 2019). In *P. ramosa*, *PrCYP707A1* is upregulated as soon as 30 min following exogenous treatment with *rac*-GR24, and up to 100-fold after 24 h (Lechat et al., 2012). Moreover, exogenous application of Abz-E2B, a specific inhibitor of *CYP707A* enzymes (Okazaki et al., 2012) completely abolishes germination of broomrapes and witchweeds (Brun et al., 2019). This suggests that early induction of ABA catabolism by *CYP707A* enzymes is a prerequisite for seed germination of parasitic weeds. Opposite to this is the recent demonstration that seeds of *S. hermonthica* are insensitive to 100 μ M ABA during germination (Fujioka et al., 2019). Due to the high genetic variability within and between *Striga* populations (Bozkurt et al., 2015; Unachukwu et al., 2017), these controversial results might simply be explained by the use of different genetic variants between studies. As compared to the early induction of ABA-related genes in obligate root parasites, the actual decrease in ABA content rather constitutes one of the late germination events when the radicle is about to protrude out of the seed coat (Lechat et al., 2012; Toh et al., 2012). Lechat et al. (2012) observed that early induction of *PrCYP707A1* expression occurs in two cells beneath the micropyle, which colocalizes with the putative site of SL perception (Plakhine et al., 2012). Moreover, only 58 genes are differentially expressed after 6 h of *rac*-GR24 treatment in *P. ramosa* (Lechat et al., 2012), which corroborates studies conducted in *Arabidopsis* showing that SL application does not induce massive changes at the transcript level (Mashiguchi et al., 2009). By contrast, exogenous application of *rac*-GR24 on *P. aegyptiaca* seeds for 48 h results in thousands of differentially expressed genes, among which ABA biosynthetic and GA degradation genes are downregulated, while GA biosynthetic genes are upregulated (Bao et al., 2017). These results suggest that early events of SL signaling consist of rapid and localized—yet undetected so far—ABA degradation. This would act as a signal that propagates throughout the seed and activates transcriptional responses in favor of a decrease in ABA/GA balance at a larger scale to induce germination (Figure 1).

At present, information on parasitic weed seed germination is limited and most often consists of finding similarities to major regulatory pathways found in nonparasitic species. The fact that parasitic weeds adapt to their hosts'

environment by responding to non-SL molecules raises the question of how their perception systems have evolved. Brun et al. (2019) demonstrated that DCL and 2-PEITC also trigger upregulation of *CYP707A* genes in broomrapes, as observed for *rac*-GR24 (Lechat et al., 2012). While this supposes that HTL/KAI2 proteins can also perceive DCL and 2-PEITC, it remains plausible that these are perceived by completely different receptors. In addition, exogenous application of Abz-E2B fully inhibits seed germination in broomrapes and witchweeds (Brun et al., 2019) but not in nonparasitic species like *A. thaliana* (Okazaki et al., 2012). Similarly, *Arabidopsis cyp707a* mutants, although hyperdormant, ultimately germinate under appropriate light and temperature conditions (Kushiro et al., 2004; Okamoto et al., 2006). This suggests that alternate pathways must be able to compensate the loss of function of *CYP707A* enzymes in nonparasitic species only.

Such differences between parasitic and nonparasitic species correlate with the relative insensitivity of most parasitic weeds toward cues known to influence physiological dormancy and germination of nonparasitic plants, e.g., light, nitrates, and gibberellins (Zehhar et al., 2002; Chae et al., 2004; Takagi et al., 2009; Yoneyama et al., 2013). In line with this, Bunsick et al. (2020) recently reported that the inability of *Arabidopsis* GA-depleted or GA-insensitive mutants to germinate is rescued when complemented with certain *S. hermonthica* HTL/KAI2 receptors. The authors also found that loss of *SMAX1* was sufficient to observe germination in *Arabidopsis* GA-depleted mutants, which suggests that recruitment of the HTL/KAI2 pathway and subsequent *SMAX1* degradation bypasses GA signaling, and hence GA requirement for germination, in nonparasitic species. By contrast, *S. hermonthica* seeds do contain much higher levels of HTL/KAI2 expression, and GR24-treated seeds germinate even on high concentrations of paclobutrazol, a chemical inhibitor of GA synthesis. These results are in accordance with the idea that parasitic weeds have become dependent on solely host-derived signals to germinate, while “differential wiring” (Machin and Bennett, 2020) of independent pathways allows nonparasitic species to respond to a wider range of exogenous cues. Although pioneering, this study does not explain why parasitic weeds do not germinate upon exogenous GA alone. Therefore, collection of genomic and transcriptomic data would be a major step forward in identifying the molecular mechanisms gained and lost during the evolution toward such a restricted germination process.

Haustoriogenesis—the A-P-C of haustorium development

Most molecular studies on haustoriogenesis focus on a relatively small number of species that cover less than 0.5% of the full parasitic plant species range and diversity (Clarke et al., 2019). Newly released genomes and whole transcriptome profiles of these species portray a familiar picture across different evolutionary lineages: many genes and

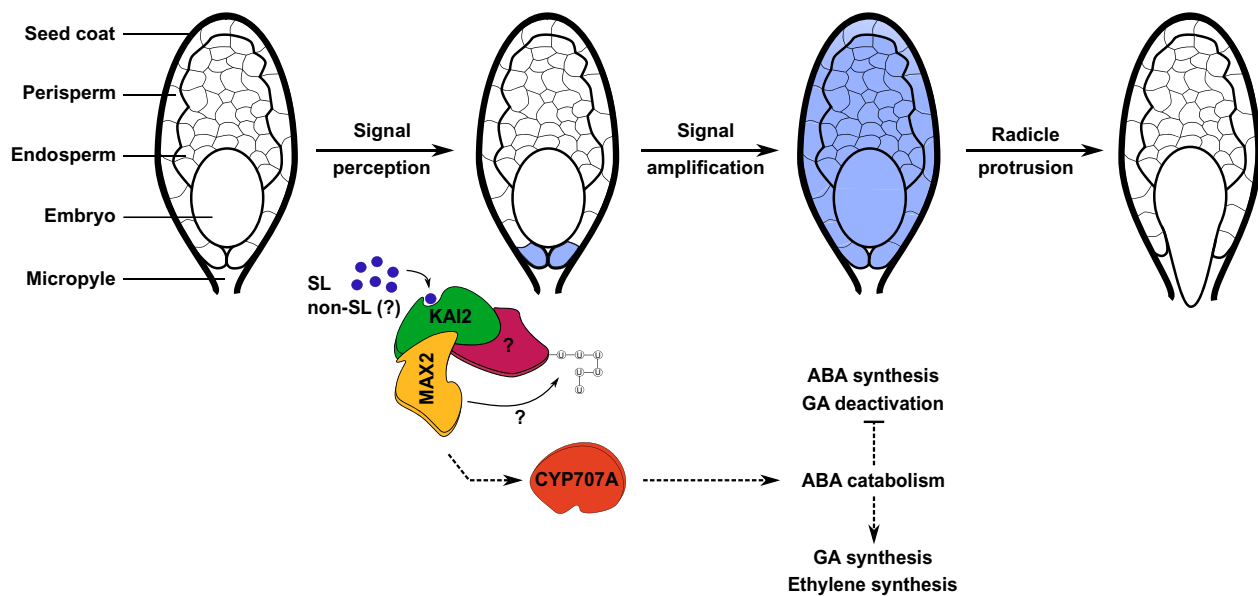


Figure 1 Hypothetical model for stimulant-induced seed germination of root parasitic weeds of the *Orobanchaceae* family. The perception of SLs or structurally divergent (non-SL) germination stimulants occurs in two cells beneath the micropyle. Perception of SL signals (and perhaps non-SL) by KAI2 proteins triggers the recruitment of the F-box protein MAX2. In turn, MAX2 supposedly ubiquitinates other proteins still unknown in parasitic plants. This signaling pathway leads to limited transcriptional responses (indicated in blue) in these two cells, among which *CYP707A* transcripts accumulate. The localized degradation of ABA through *CYP707A* enzymes at early time points is crucial for the signal to amplify throughout the seed at later time points, whereby strong transcriptional responses and extensive modulation of ABA, GA, and probably ethylene homeostasis occur. The combined decrease in ABA contents and increase in GA and probably ethylene contents enables the radicle to protrude out of the seed coat. Dashed arrows indicate unknown intermediate steps in between molecular events.

molecular pathways of haustoriogenesis are rooted in the developmental processes of their nonparasitic past (Figure 2).

Root parasites form haustoria laterally or terminally. In either case, their development is generally induced by host-related chemical signals, called haustorium-inducing factors (HIFs). The efficiency of phenolic compounds or cytokinins (CKs) as HIFs, in addition to the subsequent redox or cytokinin signaling pathways, is well established in many *Orobanchaceae* (Goyet et al., 2017; Wada et al., 2019; Billard et al., 2020). CKs are efficient in haustoriogenesis induction in many root holoparasites such as *O. cumana*, *O. aegyptiaca*, and *P. ramosa*, suggesting the loss of sensitivity to phenolics in these species during evolution toward holoparasitism. Unlike in *Orobanchaceae*, haustoria in the stem parasite *Cuscuta* (dodders) form in response to light and mechanical stimuli, indicating that the most upstream molecular events in haustorium initiation differ between *Cuscuta* and *Orobanchaceae* species. Exogenously applied CKs, however, bypasses the need for light and a mechanical stimulus to induce haustoriogenesis in *Cuscuta*, suggesting a broader role of cytokinin signaling in haustoriogenesis (Haidar et al., 1998; Ramasubramanian et al., 1988). Notably, not only plant-derived chemicals serve as HIFs. Sphaeropsidones, phytotoxins of the fungal plant pathogen *Diplodia cupressi*, are potent HIFs in *S. hermonthica*, *O. cumana*, and *O. crenata* (Fernández-Aparicio et al., 2016a).

Haustoriogenesis can be divided into at least three phases (Figure 2): (1) the Attachment to host tissue is followed by

(2) the Penetration of the host, and (3) the Connection to the host vasculature, altogether the “A-P-C” of haustorium development. Haustorial structures such as haustorial hairs, haustorial papillae, and holdfasts facilitate attachment. Interestingly, these adhesive structures share developmental mechanisms with root hairs or leaf trichomes. In *Cuscuta*, localized swellings termed holdfasts (prehaustoria) form by elongation of epidermal and cortical cells toward the host surface and adhere tightly to it (Shimizu and Aoki, 2019). Such adhesive organs also occur in root parasites, for example, in *Nuytsia* (Heide-Jørgensen, 2008). In *Orobanchaceae*, HIFs induce prehaustoria in successive steps that include a temporary (lateral haustoria) or a complete (terminal haustoria) arrest of root growth, which is then followed by localized root swellings and the formation of haustorial hairs or papillae. Like regular root hairs, haustorial hairs are filamentous single-cell protuberances of the root epidermis, while haustorial papillae are short extensions of epidermal cells. In the facultative parasites *Triphysaria* and *Phtheirospermum* (*Orobanchaceae*), haustorial hairs proliferate from the surface of the developing haustoria within 12 h after HIF application (Ishida et al., 2016; Wang et al., 2019). Blocking haustorium initiation by the coapplication of HIFs and redox inhibitors prevents the emergence of haustorial hairs, indicating that redox-regulated processes control both: the haustorial hair formation and the initiation of haustorium development (Wang et al., 2019). Similar effects were reported in *S. hermonthica* and other parasitic plants (Goyet et al., 2019; Wada et al., 2019). Downstream of HIF

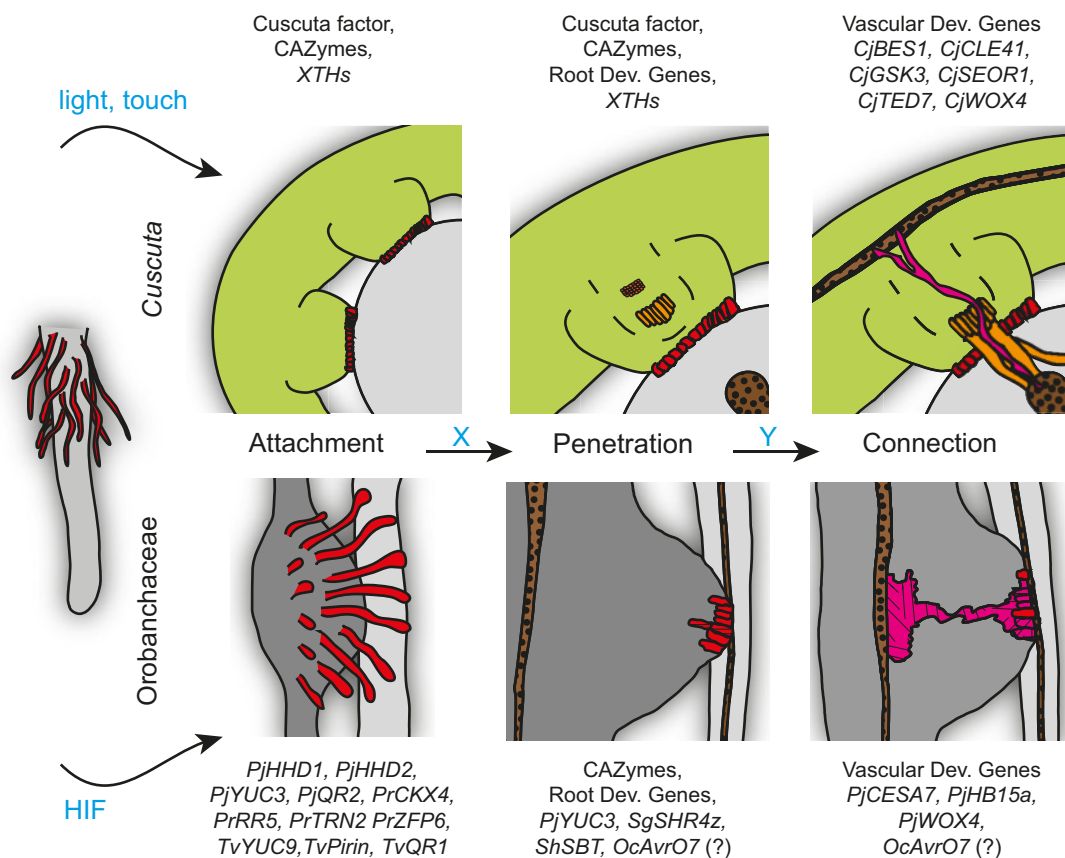


Figure 2 Checkpoints during haustoriogenesis in *Cuscuta* spp. and *Orobanchaceae*. Attachment structures (red) such as holdfasts and haustorial hairs share similarities to root hairs of nonparasitic plants (left). External factors (blue) trigger the formation of these attachment structures. Other, yet unknown, factors (X, Y) initiate the progression through the different phases of haustoriogenesis. Genes and proteins from *C. japonica* (Cj), *O. cumana* (Oc), *Phtheirospermum japonicum* (Pj), *P. ramosa* (Pr), *T. versicolor* (Tv), and *S. hermonthica* (Sh) that accompany the different phases of haustoriogenesis and that have been studied in more detail are highlighted above and below the schematic images. Vascularization of the haustoria is shown in pink, the searching hyphae of *Cuscuta* in orange. The representations of haustoria are loosely based on micrographs published in Heide-Jørgensen and Kuijt (1993); Svubova et al. (2017), and Wakatake et al. (2018). CAZymes, carbohydrate-active enzymes; Dev., developmental; XTH, xyloglucan endotransglucosylases/hydrolases.

perception, haustorial hair formation and haustorium development diverge, as *Phtheirospermum japonicum* haustorial hair defective (*hhd*) mutants form wild type like haustoria (Cui et al., 2016). The absence of root hairs in *Phtheirospermum* *hhd* mutant shows that once initiated, haustorial hair formation depends on genes that also regulate root hair formation outside the haustorium surface. Knowledge is much lower in the obligate root parasites, mainly due to the absence to date of mutants affected in haustoriogenesis. In *P. ramosa*, haustorial papillae formation, which becomes evident by the proliferation of cells at the tip of germinating seeds, is similar in its kinetics to facultative parasites (Goyet et al., 2017). In addition, blocking haustoriogenesis by the coapplication of HIFs and an inhibitor of cytokinin perception, PI-55, prevents the HIF-induced root growth arrest and the formation of haustorial papillae, indicating that CKs control both. As a result, PI-55-treated *P. ramosa* seeds are less aggressive toward host roots.

Host attachment mediated by haustorial hairs or papillae in the *Orobanchaceae* and by epidermal cells of holdfasts in

Cuscuta goes beyond a mechanical clasping of the host plant. These specialized cells also produce adhesive substances that further facilitate close adherence to the infected host organ (Yoshida et al., 2016). In *Cuscuta*, mechanical stimuli induce the secretion of polysaccharides and cell wall degrading enzymes, even in the absence of a living host (Olsen and Krause, 2017). The activity of cell wall degrading enzymes such as xyloglucan endotransglucosylases/hydrolases (XTHs) outlines the invading *Cuscuta reflexa* haustorium (Olsen and Krause, 2017). Chemically inhibiting XTH activity blocks penetration, thus arguing for the importance of cell wall degrading enzymes in aiding endophytic growth of the parasite (Olsen and Krause, 2017). Concurrently, transcriptomes of *C. australis* prehaustoria are overrepresented by genes linked to lignin and xyloglucan metabolism (Sun et al., 2018; Vogel et al., 2018). Likewise, ~20% of identified carbohydrate-active enzyme-categorized genes (CAZyme) in *S. hermonthica* show differential expression during host invasion (Yoshida et al., 2019). In particular, genes associated with pectin metabolism, typically found in primary cell walls,

are expressed in an early stage of haustorium development. The prevalence of genes linked to cell wall remodeling in early haustorium development extends to transcriptomes of other *Orobanchaceae*, *Cuscuta*, and parasitic *Santalaceae* species (Yang et al., 2015; Zhang et al., 2015; Ichihashi et al., 2018). Intriguingly, two CAZymes that are expressed in *C. campestris* haustoria show strong indications of being acquired by horizontal gene transfers early in the evolution of the *Cuscuta* genus (Yang et al., 2016).

Behind the epidermal cell layer, the *Cuscuta* prehaustoria contain disk-like meristems that locate near the vascular cylinder. Cells divide first anticlinally and then arrange into an array of digitate cells prior to tissue invasion (Svubova et al., 2017). These digitate cells develop into intrusive cells within the searching hyphae. Searching hyphae then grow endophytically through the host tissue until they reach the host vasculature. Additionally, searching hyphae may also incorporate epidermal cells, as seen for *C. europaea* (Svubova et al., 2017). In *Phtheirospermum*, intrusive cells transdifferentiate from epidermal root cells (Wakatake et al., 2018). Epidermal root cells generally play a crucial role in the development of *Phtheirospermum* haustoria. With the initiation of haustorium development, epidermal cells at the haustorium initiation site express an enzyme that catalyzes the rate-limiting step in de novo auxin synthesis, *PjYUC3* (Ishida et al., 2016). Silencing *PjYUC3* expression lowers infection rates and ectopic expression of *PjYUC3* produces swellings of the root that resemble prehaustoria. Parallel to *PjYUC3* expression, the auxin response reporter DR5 is highly active in developing *Phtheirospermum* haustoria (Ishida et al., 2016). Interestingly, auxin alone triggers the expression of CAZymes in nonparasitic plants, many of which have homology to the CAZymes upregulated in prehaustorial tissue (Majda and Robert, 2018).

Given the central role of auxin in root development, it may be of little surprise that the transcriptomes of developing haustoria in *Orobanchaceae* and *Santalaceae* root parasites are similar to root expression profiles in nonparasitic plants. The root developmental program is thereby not simply blueprinted onto haustorial development. For example, *S. hermonthica* radicles infecting rice do not express the key components of the lateral root development program *ALF4* and *ACR4* (Yoshida et al., 2019). By contrast, host plants expressed *ALF4* and *ACR4* coincidentally. The relevance of parasite-induced *ALF4* and *ACR4* expression in host plants is currently not known, but it suggests intertwined signaling between parasite and host.

Similarities between haustoriogenesis and the gene expression in nonparasitic roots were also observed in *C. australis* (Sun et al., 2018). This observation is surprising as *Cuscuta* species are rootless plants that lost and pseudogenized many root-associated genes (Sun et al., 2018; Vogel et al., 2018). To which extent *Cuscuta* haustoriogenesis interconnects to host responses has also been a continuous subject of interest.

An emerging aspect of these studies is the question of how parasitic plants avoid detection by the host immune

system. When parasitic plants trigger an elevated host immune response, haustoriogenesis aborts, and haustoria degenerate. For example, in tomato (*Solanum lycopersicum*), the plasma membrane receptor CuRe1 recognizes a *Cuscuta* conserved peptide factor (Hegenauer et al., 2016). Recognition of the *Cuscuta* factor triggers a robust immune response, visible as brownish dead tissue around the penetration. Similarly, the recently cloned HAOR7 receptor-like kinase in sunflower detects AvrO7-containing *O. cumuna* races (Duriez et al., 2019). HaOR7-induced resistance leads to termination of the infection process when *O. cumuna* penetrates the outer sunflower root layers and prevents its connection to the host vasculature. *S. hermonthica* haustoria also fail to connect to the vasculature of resistant rice varieties (Cissoko et al., 2011). Overcoming resistance to establish parasitism successfully is a matter of life and death for obligate parasites and likely involves effector proteins as recently shown for *S. gesnerioides* (Su et al., 2019), also highlighted in Delavault's commentary (2020).

For parasitic plants that slip through host detection, haustoria mature and enclose “the most general anatomic feature of the haustorium”—the xylem bridge (Heide-Jørgensen, 2008). Xylem bridges comprise lignified xylem vessels that span the entire haustorium and connect parasite to host vasculature. The (pro-)cambium orchestrates vascular development in plants (Jouannet et al., 2015). (Pro-)cambial-like domains within the haustorium cortex express cambial marker genes such as *CLE41*, *GSK3*, *BES1*, and *WOX4* in *C. japonica* (Shimizu et al., 2017). The expression of these marker genes suggests that the default vascular development program is also active in maturing *Cuscuta* haustoria. Unlike in nonparasitic plants, where phloem cells express and secrete *CLE41* and cambial cells respond to the perceived *CLE41* peptide by expressing *CjWOX4*, *Cuscuta* haustoria show overlapping *CjCLE41* and *CjWOX4* expression domains. The authors speculate that the lack of distinct *CLE41* and *WOX4* expression domains points at additional host cues that could participate in the organization of the vasculature in *Cuscuta* (Shimizu et al., 2017).

In *Phtheirospermum*, (pro)cambial-like cells within the haustorium core express *PjWOX4* in a similar temporal and spatial manner to *Cuscuta* haustoria (Wakatake et al., 2018). *Phtheirospermum* (pro)cambial-like cells transdifferentiate from endodermal, cortical, and epidermal cells, but not from procambial stele tissue of the main root. The de novo formation of the haustorial (pro)cambium further supports a more than hundred-year-old conclusion by Stephens that haustoria are not modified lateral roots, but “organs sui generis” (Stephens, 1912).

CKs play another critical role in vascular development. CKs promote radial growth through periclinal divisions (De Rybel et al., 2015). CKs increase during the transition from pre- to mature haustoria in different lineages of parasitic plants (Zhang et al., 2012; Furuhashi et al., 2013; Spallek et al., 2017). In *Phtheirospermum*, CK responses occur not only in (pro-)cambial tissue of the haustorium cortex but

also in intrusive cells at the haustorial apex (Spallek et al., 2017). The proximity of intrusive cells to host vessels allows the transfer of parasite-derived CKs to the host to trigger hypertrophy in host plants.

Hemiparasitic plants only connect through xylem bridges to the host vasculature. Holoparasites such as *Cuscuta* and broomrapes, by contrast, also withdraw phloem-mobile molecules from their host. Phloem structures within haustoria are far less distinctive compared to xylem vessels of xylem bridges. *Cuscuta* haustoria express phloem companion marker genes such as *CjAPL* and *CjSEOR1* (Shimizu et al., 2017). Still, no phloem companion cells were detected adjacent to cells that symplastically transported a fluorescent dye that was fed to the host. Also, *P. aegyptiaca* phloem conducting cells differ from regular phloem conducting cells in that they contain nuclei (Ekawa and Aoki, 2017). Of particular interest is the interface between host and parasite phloem. Electro-microscopic studies of *O. cumana* infecting sunflower roots show direct sieve-element connections and share interspecies sieve plates. Interspecies sieve plates show an uneven callose deposition, with more callose on the host side (Krupp et al., 2019). *O. cumana* sieve-tube elements connected to sunflower sieve-tubes lack nuclei and vacuoles and are accompanied by companion cells, thus resembling sieve elements-companion cell complexes of nonparasitic plants. The differences in phloem morphology, even within the *Orobanchaceae*, further support the need to study a broader range of diverse parasitic plant species at cellular resolution, using, among others, methods that have been previously successfully employed to analyze parasitic plants–host interactions (Honaas et al., 2013).

Concluding remarks and perspectives

Overall, in comparison with many other organisms, the progress of knowledge in the biology of parasitic plants, especially obligate root parasites, has been limited until recently mainly due to the lack of data on their genetics. In 2018, Vogel et al. and Sun et al. sequenced for the first time the genomes of field dodder (*C. campestris*) and Australian dodder (*C. australis*), respectively. In 2019, Yoshida et al. published the complete genome sequence of the witchweed *S. asiatica*. The genomes of two broomrape species, *P. ramosa* and *O. cumana* (<http://www.heliagene.org>), are in the process of finalization. In addition, several transcriptomic datasets are already available for *P. japonicum*, *P. ramosa*, *P. aegyptiaca*, *S. gesnerioides*, *S. hermonthica*, and *T. versicolor* (Yang et al., 2015; Ishida et al., 2016; Goyet et al., 2017; Yoshida et al., 2019). Combined, these datasets provide the raw material for future studies that must now employ approaches to validate the function of key candidate genes involved in critical processes of parasitic plant development. At present, several transformation protocols have been implemented in *Triphysaria versicolor* (Tomilov et al., 2007), *P. aegyptiaca* (Fernández-Aparicio et al., 2011), *P. japonicum* (Ishida et al., 2011), *S. hermonthica* (Kirigja et al., 2014), and *P. ramosa* (Libiaková et al., 2018). However, there is still a

OUTSTANDING QUESTIONS

- What are the molecular actors responsible for and subjected to extensive epigenetic remodeling during the conditioning period?
- What genomic reconfigurations underlie the acquisition of a seed germination process that is strictly dependent on perception of host-exuded signals in parasitic weeds?
- Is the ability to germinate upon non-SLs signals driven by the neofunctionalization of HTL/KAI2 proteins or the acquisition of unique perception pathways?
- Which host-derived signals control haustoriogenesis and how are they perceived?
- Which parasite gene products are recognized by host immunity and which ones evolved to subvert it?
- How can we produce stable transgenic stem and root parasitic plants?

long way to go before being able to generate stable transgenic obligate parasitic plants, and thus before applying high-throughput reverse genetics. Yet, this remains a crucial step in the objective of developing methods to control parasitic weeds. Although several physical, cultural, chemical, and biological approaches have been explored (Fernández-Aparicio et al., 2016b), none of them has proven unequivocal success. The fact that the root parasitic weed withers its host long before it emerges aboveground suggests that the early stages of its development are interesting targets to design control strategies. This has become even more relevant in recent years, with the identification of key molecular components involved in parasitic weeds recognition of host-derived chemicals required for seed germination and haustorium formation.

In the Outstanding Questions Box, we present some examples of challenges faced in deciphering the biology of parasitic plants. Answers to these questions will fill important knowledge gaps and can be used to develop new strategies for controlling parasitic plants.

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