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Review paper

Transcriptomics exposes the uniqueness of parasitic plants

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Abstract

Parasitic plants have the ability to obtain nutrients directly from other plants, and several species are serious biological threats to agriculture by parasitizing crops of high economic importance. The uniqueness of parasitic plants is characterized by the presence of a multicellular organ called a haustorium, which facilitates plant–plant interactions, and shutting down or reducing their own photosynthesis. Current technical advances in next-generation sequencing and bioinformatics have allowed us to dissect the molecular mechanisms behind the uniqueness of parasitic plants at the genome-wide level. In this review, we summarize recent key findings mainly in transcriptomics that will give us insights into the future direction of parasitic plant research.

Key words: parasitic plants; transcriptomics; haustorium; plant–plant interaction; photosynthesis

Introduction

Parasitic plants acquire water, carbon and nutrients via vascular connections to the host plants [1, 2]. Parasitic plants consist of ~4000 species from 19 different families that can be found in most major biomes [3]. They can be classified based on their life cycle and mode of nutrition: (i) dependence on host: a parasite that requires a host to complete its life cycle is termed as 'obligate', a parasite that does not is termed as 'facultative'; (ii) presence or absence of chlorophyll: partially photosynthetic are termed as 'hemiparasitic', and non-photosynthetic as 'holoparasitic'; (iii) points of attachment: root or stem parasites [4]. Parasitism in angiosperms has originated independently at least 11 times [5], which is an example of convergent evolution.

Parasitic plants develop a multicellular organ called a haustorium, whose functions include attachment and invasion to a host and the physiological redirection of host resources into the parasite [2]. Haustorium formation and seed germination occur in response to host-derived chemical cues [2, 6]. Parasites and host plants exchange mobile molecules through the haustorium [7] and exhibit defense response at the haustorial interface in the root tissues, such as root endodermis, cortex and epidermal layers, which are in contact with the host [8, 9]. In extreme cases of plant parasitism (holoparasite), the parasites have lost their chloroplasts and are unable to carry out photosynthesis [10]. Thus, the uniqueness of parasitic plants is characterized by the development of the haustorium that enables nutrient acquisition and reduction of photosynthesis, contrasting with

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autotrophy of typical plants that produce organic nutrients through photosynthesis [2].

Several species of parasitic plants have severe effects on host plant growth and have a serious economic impact on agriculture [2, 4, 11]. Since agriculture began ~10 000 years ago, the invasion of parasitic plants from tropical grasslands to agricultural lands has occurred within a relatively short period [12]. For example, *Striga*, *Alectra* and *Orobanche* infect a wide range of plants in the dry tropics and subtropics. In Africa, five of the most economically important *Striga* species, such as *Striga hermonthica*, *Striga asiatica*, *Striga forbesii*, *Striga aspera* and *Striga gesnerioides*, affect the production of sorghum, millet, maize, sugarcane and cowpea resulting in losses of up to USD 1 billion annually affecting >100 million farmers [4, 11]. Moreover, hundred thousand hectares in Europe, Russia, Middle East, China, Cuba and parts of the USA are infested with *Orobanche* species [13].

Efforts to control destructive parasitic plants such as *Striga* and *Orobanche* have been hampered by the complicated association between the parasites and their hosts. Several control methods, including crop rotation, hand-pulling, biological control using *Fusarium oxysporum* [14] and chemicals such as ethylene gas [15], have been tried, but their effectiveness is limited by the size of infested areas, inefficient delivery systems and costs. Therefore, development of crops resistant against these hazardous parasitic plants is proposed to be the only viable strategy [2]. Understanding the molecular mechanism allowing parasitic plants to parasitize host plants is an important step toward generating new resistant crops. Combining advanced technologies such as next-generation sequencing and bioinformatics has allowed us to perform genome-wide gene expression analysis (transcriptomics) on non-model organisms to investigate the molecular mechanisms of host–parasite interactions [16]. In this review, we highlight recent key findings on parasitic plants obtained from transcriptomics studies and the unique aspects that allow parasitic plants to support their heterotrophic life cycle.

Haustorium

What is the haustorium of parasitic plant?

The haustorium is a key multicellular organ shared across all parasitic plants, providing physical and physiological bridges between hosts and parasites (Figure 1A) [17]. Root parasites such as *Orobanchaceae* species have a terminal/primary haustorium, which develops on the tip of a primary root soon after germination, and also a lateral/secondary haustorium, which forms laterally on the growing roots without loss of root tip growth. These two types of haustoria show similar anatomic structures and physiological roles. During haustorial development, cells in the tip or lateral side of the root start expansion and division making a globular-shaped organ [18–20]. Concomitantly, the epidermal cells differentiate to hair cells, recognized as haustorial hairs [21]. After penetration into host tissue, host and parasite vessels are connected via the so-called 'xylem bridge' (Figure 1B) [22]. A 'hyaline body' occupies the central haustorial parts surrounding the xylem cells. This hyaline body has distinctive parenchymatous cells with dense cytoplasm and characteristic extracellular deposits, which may have a function associated with nutrient translocation [23, 24]. Anticlinally elongated epidermal cells, designated as palisade cells, line up at parasite–host interface of the haustorium [25]. *Striga* species also develop a specialized structure called 'oscula', which intrusively penetrates into host vessel elements to absorb water and nutrient [26].

By contrast, the stem parasite *Cuscuta* has only rudimentary root system, which disappears a few days after germination and is replaced by haustoria, which develop from the differentiated stem. Development of a haustorium is preceded by a prehaustorium whose initiation is manifested by cell division at sites adjacent to the vascular bundle. In this prehaustorium stage, the vascular proximal cells are dividing, while the tip of the prehaustoria has a zone of large elongated cells, eventually differentiating into searching hyphae [27]. The searching hyphae transforms into xylic or phloic structures depending on the cell types that hyphae encounter on host penetration [28]. Thus, similar but distinct structures underpin the parasitic functions of haustoria in root and stem parasites.

Most facultative parasites are generalists in host specificity, but some obligate parasites only parasitize a limited range of host species [29]. For instance, *S. asiatica* and *S. hermonthica* parasitize only monocots in natural conditions. Nevertheless, they can penetrate but not completely parasitize eudicots, whereas *S. gesnerioides* parasitize eudicots, especially cowpea. Given the different types of incompatibilities shown in interactions between *S. hermonthica* and nonhost species, it is likely that host range is determined by multiple mechanisms [9]. Interestingly, *S. hermonthica* rarely forms haustoria when germinated alongside *Phtheirospermum japonicum*, a facultative parasite in Orobanchaceae, suggesting the existence of mechanisms for recognizing closely related parasitic species [9].

Auxins functions in haustorium

Auxins are crucial in the development of almost all organs of plants [30]. Exogenous application of auxin positively affects haustorium numbers, while disturbing auxin flux using auxin efflux or activity inhibitors results in reduction of haustorium number in *Triphysaria versicolor* and in *Phelipanche aegyptiaca* [31, 32]. Series of root tip dissection experiments have demonstrated that local auxin accumulation is involved in haustorium initiation in *T. versicolor* [32]. Expression of auxin responsive *IAA2* promoter in hairy root of *T. versicolor* is further evidence of this local auxin accumulation [32]. A transcriptome study in the stem parasite *Cuscuta pentagona* revealed that genes associated with polar auxin transport activity are enriched in the haustoria stage as compared with stems or seedlings [33]. These data suggest that auxin maxima are established to induce haustoria formation in both root and stem parasites. In addition to auxin, an ethylene response is detected in *T. versicolor* [32] and gibberellic acid- and strigolactone-related genes are upregulated in the haustorium of *C. pentagona* [33]. These observations imply cross talks among multiple phytohormones in haustorial development.

Haustorial genes

Currently, genes functioning in haustoria of parasitic plants are being discovered by use of molecular genetics tools in combination with transcriptomics. For example, transcriptomes combined with laser capture micro-dissection approaches successfully identified gene expression in haustorial cells in *T. versicolor* [34]. *Triphysaria versicolor* is a generalist parasite with a wide host range [29]. Comparison of gene expression in the *T. versicolor* haustoria penetrating different hosts, such as the monocot *Zea mays* and the eudicot *Medicago truncatula*, identified gene sets that are expressed commonly and differently between

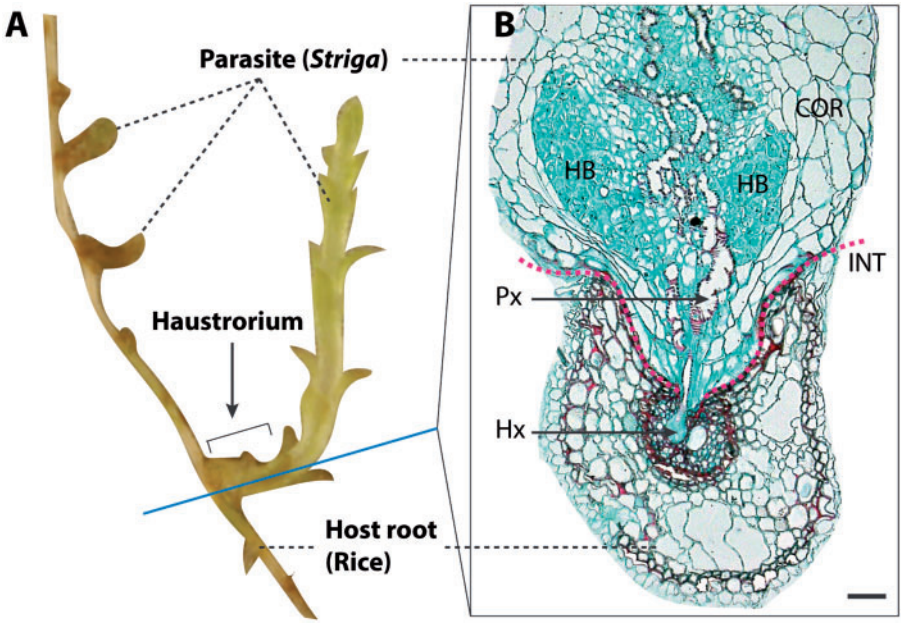

Figure 1. Haustorium of parasitic plant. (**A**) *Striga hermonthica* parasitizing rice roots. (**B**) Cross section of *S. hermonthica* haustorium penetrating rice root. The section was stained with Safranin O and Fast Green. Px, parasite xylem; Hx, host xylem; HB, hyaline body; COR, cortical parenchyma; INT, interface between the haustorium and the host root. Bar is 500 μm. (A colour version of this figure is available online at: http://bfg.oxfordjournals.org)

host species. Although ~30% of genes highly expressed at the host–parasite interface are unknown, *Expansin* genes are expressed in haustorial cells in a host-specific manner [34]. Expansins act nonenzymatically to loosen cell walls; β-expansins have an activity specific to grass species, whereas α-expansins target eudicots and other monocots [35–37]. Consistent with their protein functions, expression of β-expansin is higher in the *T. versicolor*–infecting *Z. mays* as compared with that in *T. versicolor*–infecting *M. truncatula* and α-expansin expression levels are similar in both interactions. These results suggest that the generalist parasites may leverage distinct genes to parasitize different host plants.

Alakonya *et al.* [38] found that the *C. pentagona SHOOT MERISTEMLESS-like* (*CpSTM*) gene is highly expressed at prehaustorial stage in *C. pentagona* with a tobacco host. Silencing signals of small interfering RNA (siRNA) can be transmitted through parasite–host attachment as shown in root parasites [39, 40]. Using the cross-species movement of siRNA revealed that knocking down of *CpSTM* disrupts dodder growth by inducing defects in haustorium connection, development and establishment as well as misdirecting growth of searching hyphae [38]. As *STM* is known to regulate shoot development in other plant species [41], this finding contrasts previous hypothesis that dodder haustorium has root origin. Thus, involvement of *CpSTM* in haustorium development programs suggests that the haustorium has a mixed nature of shoot and root origin by co-opting both developmental programs.

Plant–plant interaction

Recognition of host

It has been known for many years that Orobanchaceae plants generally develop haustoria only when grown in the presence of other plants (Figure 2A) [2]. The first chemicals identified as haustorium-inducing factors were the flavonoids xenognosin A and xenognosin B [42], while the first haustorium-inducing factor isolated from actual host roots is 2,6-dimethoxy-p-benzoquinone (DMBQ) [43]. Biochemical analysis of DMBQ and its analogs suggests that semiquinone intermediates, formed during redox cycling between quinone and hydroquinone states, initiate haustorium development [44]. Quinone redox changes are catalyzed by quinone oxidoreductases. Quinone oxidoreductases *TvQR1* and *TvQR2*, which were isolated from the facultative parasite *T. versicolor*, are upregulated after treatment with DMBQ and other quinones [45]. RNA interference experiments show that *TvQR1* is necessary for haustorium development [46]. Given that the TvQR1 enzyme is in the ζ-crystallin family of quinone oxidoreductases [46], the haustorium signaling system could be redox-activated by radicals produced by the TvQR1 reaction with haustorium-inducing factors. Transcriptomics on DMBQ treatment identified genes transcriptionally regulated by haustorium-inducing factors including *TvQR1/2* [47]. Among the identified genes, *TvPirin*, which is an ortholog associated with various environmental signaling [48, 49], was shown to be necessary for haustorium development in *T. versicolor* [50]. Furthermore, a population genetic study revealed that *TvQR1* exhibited remarkably higher molecular diversity and more recombination events than *TvPirin*, suggesting that *TvQR1* and *TvPirin* might have evolved highly distinct roles for haustorium formation [51]. Taken together, these findings point to the current model for haustorium initiation where parasitic plants trigger the host tissue to produce DMBQ, and the derived DMBQ then induces a parasite quinone oxidoreductase, which converts DMBQ into the active single-electron free radical form with the suitable redox potential for haustorium induction (Figure 2A). Notably it is known that DMBQ can induce haustorial formation in *Triphysaria* species, *Striga* species, *Agalinis purpurea* and *P. japonicum* [19, 43–45, 52, 53]. More detailed stage-specific transcriptomics will allow us to unveil the gene regulatory network that operates during haustorium formation under DMBQ activation.

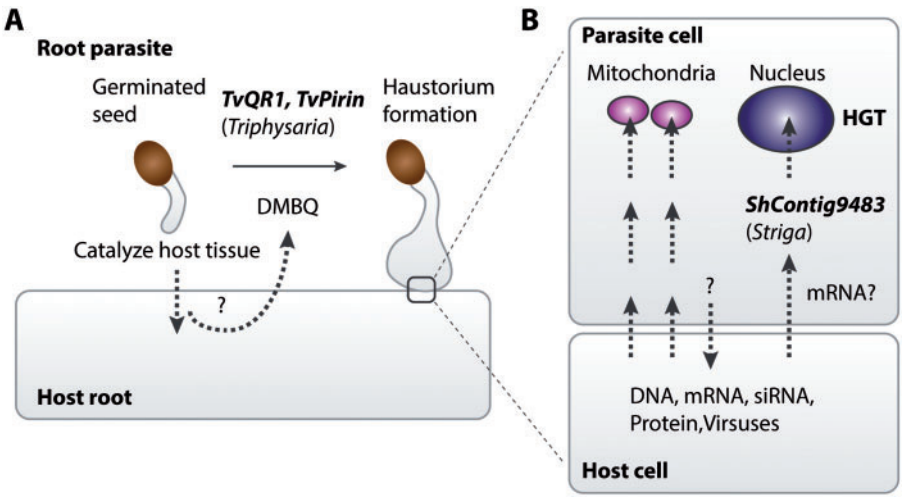

Figure 2. Parasite–host interactions. (**A**) Model for root parasite–host interactions to complete parasitizing. (**B**) Model for macromolecule trafficking between parasite and host cells. Because data were obtained from different root parasites and host plants, plant names are indicated in parentheses for each gene/pathway/transcript.

Exchange of molecules

Because parasitic plants directly connect to host plants through a continuous vascular system (and plasmodesmata in some species like *Striga* and *Cuscuta* [54–56]), movement of molecules between these plants has been reported (Figure 2B) [7]. Currently, there is evidence that parasitic plants acquire various types of macromolecules, such as DNA [5, 57–64], mRNA [65, 66], siRNA [38–40], protein [54, 67, 68] and also viruses [54, 69, 70], from their host plants. Interestingly, transcriptome analysis using *Cuscuta* and its hosts *Arabidopsis* and tomato revealed 'genomic-scale' exchanges of mRNA between parasitic and host plants [71]. The uptake and distribution of *Arabidopsis* mRNA into *Cuscuta* varies depending on the mRNA under investigation, indicating multiple routes of RNA trafficking or selective mechanism for mobile mRNA [72]. These findings raise the possibility that the movement of non-nutrient solutes may function physiologically and ecologically in parasite–host interactions [73]. For example, movement of solutes may create osmotic pressure to take up water. In addition, as siRNA can be moved between parasite and host plants [39, 40], it is possible that exchange of small RNA is involved in regulation of gene expression in both parasite and host at the transcriptional level by DNA methylation, as well as at the posttranscriptional level by direct mRNA interference [74]. A combination of detailed transcriptomics, bioinformatics and functional analyses could be a powerful tool to assess whether parasitic plants have evolved mechanisms to exchange molecules to their advantage.

In the course of evolution, nucleic acids were not only transferred from host plants but also inserted into the parasite genomes (Figure 2B). This is termed as horizontal gene transfer (HGT) and is defined as the movement of genetic material between species other than by descent. HGT of both mitochondrial and nuclear genes are reported in parasitic plants [2, 57], although HGT of mitochondrial genes might be much higher than that of nuclear genes [58]. Interestingly, a nuclear gene in *Striga*, which was derived from grass via HGT, was discovered to lack introns and has the remnants of a poly-A sequence, suggesting that the transfer occurred trough an mRNA intermediate [57]. Transcriptome data capturing the dynamics of mRNA can be used for phylogenomic approaches to identify HGT at the system level. Using such an approach, Xi *et al.* [59] examined transcriptome data of both the parasite (*Rafflesia*) and its host (*Tetrastigma*) to reveal the unidirectional host-to-parasite gene transfers. *Rafflesia* HGTs represent a wide range of cellular functions including respiration, metabolism and protein turnover, and were expressed at levels comparable with vertical gene transfer (VGT) [59]. This indicates that HGT in parasitic plants is not just a by-product of molecule movement through vascular connection but might be mechanically and biologically meaningful. Notably, VGT in *Rafflesia* shows host-like codon usage properties compared with their closest relatives [59]. This could be interpreted as suggesting that VGT may have evolved convergently to match the translational requirements of their host, thus making a suitable cellular environment for promoting and/or maintaining HGT in the parasitic plant [59]. Recent study revealed that *P. aegyptiaca* and related parasitic species and even distantly related parasitic species *C. pentagona* have obtained *albumin 1 KNOTTIN-like* genes from legumes through separate HGT events [75]. This suggests that certain genes may have been repeatedly captured by parasitic plants.

Loss of photosynthesis

Although hemiparasites still carry out photosynthesis to some extent, holoparasites completely rely on the host producing carbohydrates. In holoparasites, therefore, photosynthesis-related genes are no longer required, and may become pseudogenes and be deleted, resulting in a functional and physical reduction of the plastid genome [5, 10, 76–78]. Despite the fact that ~90% of photosynthesis-related genes are in the nuclear genome [79], the nuclear genome and transcriptome remain largely unexplored to date. Most recent advances in developing high-throughput RNA-seq allow us to readily generate the transcriptome data for multiple tissues and species [80]. These technical advances were used for multiple species in the parasitic plant family Orobanchaceae [81, 82] and also for different stages of the whole life cycle of *C. pentagona* [33]. The Parasitic Plant Genome Project has sequenced transcripts from three parasitic species, including facultative parasite *T. versicolor*, obligate hemiparasite *S. hermonthica* and obligate holoparasite *P. aegyptiaca*, and a nonparasitic relative in the Orobanchaceae [82]. Bioinformatic analysis of transcriptomes generated from the above-ground

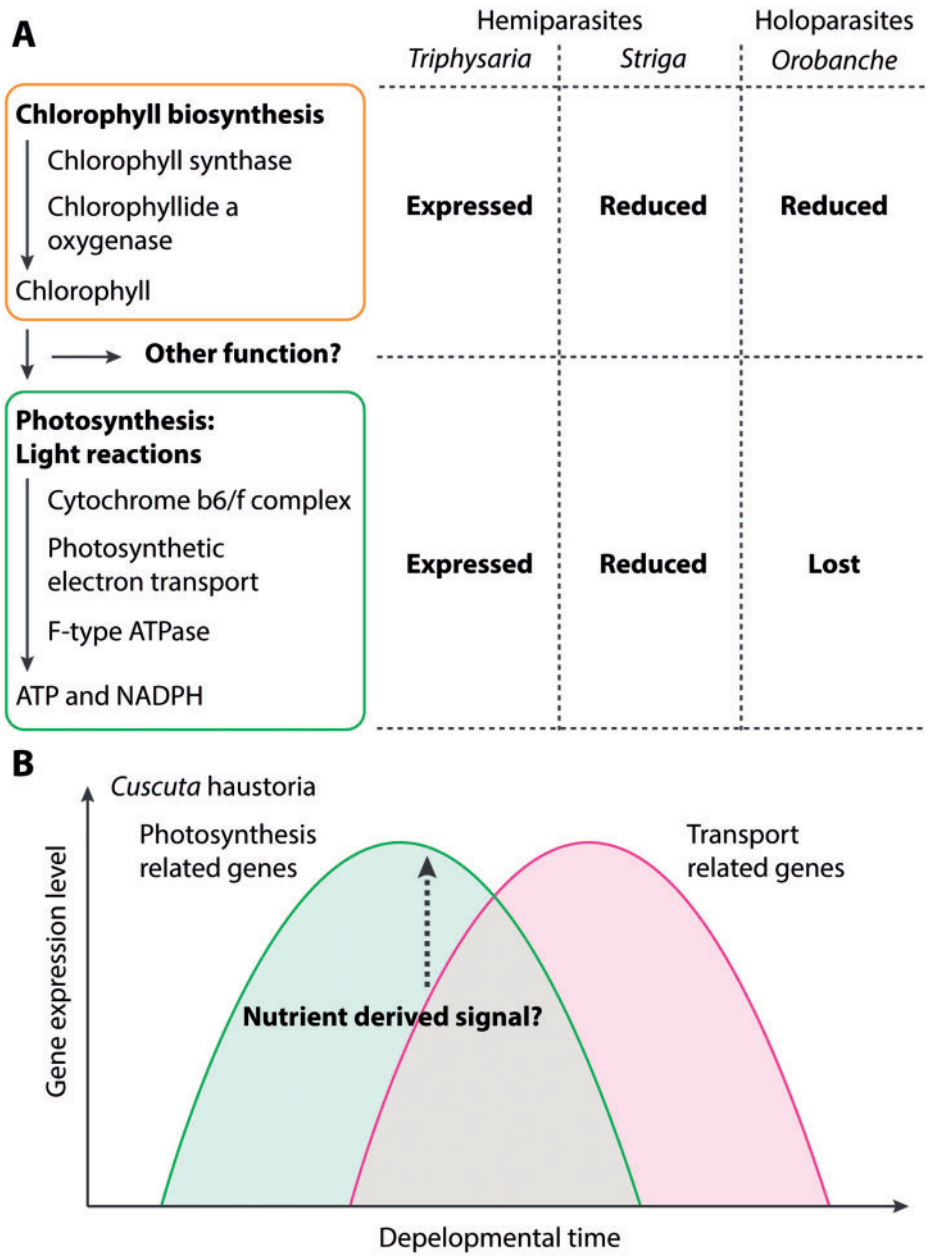

Figure 3. Loss of photosynthesis. (**A**) Expression of chlorophyll biosynthesis and photosynthesis-related genes in hemi- and holoparasites. Despite the absence of photosynthesis in holoparasite *Orobanche*, they still express intact genes in chlorophyll biosynthesis pathway. (**B**) Developmental expression patterns of photosynthesis- and transport-related genes in *Cuscuta* haustoria. The complementary expression pattern indicates that nutrients obtained from the transport activity might stimulate the decrease in expression of genes related to photosynthesis.

tissue samples reveal that the holoparasite *Orobanche aegyptiaca* lost expression of photosynthesis-related genes but surprisingly retains an intact expressed and selectively constrained chlorophyll synthesis pathway [81]. This raises an interesting question regarding the role of chlorophylls in the holoparasite, especially if chlorophylls are being produced but are not participating in photosynthesis (Figure 3A). The chlorophyll intermediates, like protochlorophyllide, may be involved in signaling rather than photosynthesis [81]. Another holoparasite *Cuscuta* also shows overall low expression of photosynthesis-related genes [33]; however, detailed transcriptomics using different developmental stages of *Cuscuta* revealed the temporal regulation of photosynthesis-related gene expression, where there is increased expression of genes related to transporter activity and reduced expression of genes related to photosynthesis with progression of plant parasitism [33]. This suggests that after successful parasitism, *Cuscuta* acquires its nutrients from the host plant mostly through haustorial transport, and reduces photosynthesis to a minimal level (Figure 3B). These findings are consistent with the characterization of plastid genomes that show gene losses and increased substitution rates in parasitic plant plastid genomes [5, 10, 76–78]. Detailed analysis, for instance, using comparative transcriptomics with close relatives [69, 78, 83], could reveal the process by which the photosynthesis machinery has been lost during evolution.

Future directions

Technologies of RNA-seq and bioinformatics analyses have revealed the molecular mechanisms underlying the uniqueness of parasitic plants such as evolving a haustorium, modifying the plant–plant interactions and shutdown of photosynthesis. Technical advances in transformation have also facilitated research in parasitic plants. Transient transformation method for facultative parasites *P. japonicum* [19] and *T. versicolor* [84] and virus-induced gene silencing for *S. hermonthica* [85] have been developed and used for functional analysis. Using transgenic host plants to silence genes in parasitic plants is a distinctive approach in the study of parasite–host interaction that can be applied for improving crops [40, 86]. High-throughput transformation technique using hairy root transformation of host plants is also useful to investigate the function of genes in hosts responding to parasitic plants [87]. However, the stable and heritable transformation, which is necessary for performing detailed molecular genetics of parasitic plants, has not been established yet.

There still remain key questions for the current parasitic plant research field: What determines the uniqueness of parasitic plants? As certain genes show unique characteristics in molecular evolution rate [88], do parasitic plants have unique genome properties to evolve their uniqueness? Or as current evolutionary developmental biology suggests [89], do parasitic plants have conventional genome properties but co-opt preexisted toolkit genes to evolve their uniqueness? To answer these questions, genome sequencing of parasitic plants is necessary in addition to the transcriptomics reviewed in this article. Sequencing parasitic plant genomes and comparing them with those of nonparasitic plants should allow us to determine the extent of conservation of gene content, genomic sequences and other genomic information, including 'functional elements' such as non-protein-coding loci, transcription start sites, regulatory sequences, chromatin accessibility and histone modification patterns [90–92] in the parasitic plant genomes. In addition, as parasitism has evolved independently >11 times [5], there may be a common molecular mechanism for parasitism in angiosperms. Interestingly, recent studies reveal that independent lineages have leveraged similar molecular pathways in the convergent evolution seen in electric organs of vertebrates and bioluminescent organs of squids [93, 94]. Similar to this case, there might be a few key genetic components that can induce parasitism in the ancestral angiosperm genome. We believe future comparative genomics across phylogenetically distant parasitic plants may reveal the answer to this question. These future functional genomic studies on parasitic plants will provide new concepts for understanding the evolutionary novelty and heterotrophic ability of parasitic plants. By extension, this will allow us to improve agricultural crops as well as solve the agricultural/economic problem of damage by parasitic plants.

Key points

- Uniqueness of parasitic plants is characterized by evolving a haustorium, which facilitates plant–plant interactions, and reducing own photosynthesis.
- Transcriptomics with cellular resolution identified key genes functioning in the haustorium.
- Transcriptomics demonstrated 'genomic-scale' exchanges of mRNA between parasitic and host plants.
- Transcriptomics revealed the process by which the photosynthesis machinery has been lost in the course of evolution.

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