Host-parasite tissue adhesion by a secreted type of β-1,4-glucanase in the parasitic plant Phtheirospermum japonicum

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Tissue adhesion between plant species occurs both naturally and artificially. Parasitic plants establish intimate relationship with host plants by adhering tissues at roots or stems. Plant grafting, on the other hand, is a widely used technique in agriculture to adhere tissues of two stems. Here we found that the model Orobanchaceae parasitic plant Phtheirospermum japonicum can be grafted on to interfamily species. To understand molecular basis of tissue adhesion between distant plant species, we conducted comparative transcriptome analyses on both infection and grafting by P. japonicum on Arabidopsis. Despite different organs, we identified the shared gene expression profile, where cell proliferation- and cell wall modification-related genes are up-regulated. Among genes commonly induced in tissue adhesion between distant species, we showed a gene encoding a secreted type of β-1,4-glucanase plays an important role for plant parasitism. Our data provide insights into the molecular commonality between parasitism and grafting in plants.
Exceptionally in the autotrophic plant lineage, parasitic plants have evolved the capability to absorb water and nutrients from other plants. This ability relies on a specialized organ called a haustorium, which forms a physical and physiological connection between the parasite and host tissues. Plant parasitism has independently evolved in angiosperm lineages at least 12 times and ~1% of angiosperms are estimated to be parasitic. Among these species, the Orobanchaceae family is the most species-rich and includes the notorious agricultural pests Striga spp., Phelipanche, and Orobanche spp., which threaten world food security.

The infection process of parasitic plant to host plant tissues is initiated with physical contact between the parasitic haustorium and the host tissue. After recognizing the host plant by the acceptance of host-derived compounds, the parasitic plants promote the development of haustorium and haustorial hairs derived from epidermal cells. Then, adhesion between parasitic haustorium and host plants is established. In the haustorial hair defective mutants, the number of haustoria formed upon infection of the host roots is reduced, but the internal structure of haustoria remains intact. Electron micrographs of the interaction between Striga haustorium and a host show that parasitic invasion is accompanied by host cell wall alterations, but not disruption, such as partial wall dissolution and shredding. Similarly, Orobanche spp. penetrate the host root tissues where pectolytic enzyme activity is evident around haustoria. Activities of cell wall-degrading enzymes, such as cellulase and polygalacturonase, are also present in infecting Phelipanche tubers. In the case of stem parasites, such as dodder (Cuscuta pentagona), epidermal cells differentiate into secretory trichomes that excrete a cementing substance predominantly composed of de-esterified pectins, and the cell walls are modified by a cell wall-loosening complex. The parasitic haustorium thus is able to adhere to the host tissues either in roots or in stems.

All the parasitic plants known to date are able to establish vasculature connection to host, which can be considered as "natural grafting". Especially, one of the interesting characteristics of parasitic plants is their ability to adhere to the apoplastic cell wall matrix of phylogenetically distant plant species of diverse cell wall composition. This adhesion ability is also crucial for "artificial grafting", in which cut stem tissues are assembled to unite, and often causes incompatibility among interfamily species. In the case of compatible graft combinations, the grafted parts are connected through tissue adhesion. Compressed cell walls in the region of the graft junction have been observed during grafting, which indicates that the cell walls between opposing cells at the graft interface adhere followed by vascular reconstruction and tissue union between the grafted organs. The mechanism of how parasitic plants are able to overcome incompatibility in tissue adhesion with a diverse range of host plant species remains unclear.

To understand molecular events during parasite infection, transcriptome analyses have been conducted on several parasitic plants, including species of Orobanchaceae, as well as dodder. In particular, Yang et al. identified putative parasitism genes that are upregulated during haustorial development following host attachment in three Orobanchaceae parasitic species. Among them, genes that encode proteases, cell wall-modification enzymes, and extracellular secretion proteins are highly upregulated. Similarly, transcriptome analysis of dodder revealed increased expression of genes encoding cell wall-modifying enzymes, such as pectin lyase, pectin methyl esterase, cellulase, and expansins, in the infective stages. A transcriptome analysis of Thesium chinense, a parasitic Santalaceae plant, also identified highly upregulated genes that encode proteins associated with cell wall organization as a peripheral module in the gene co-expression network during developmental reprogramming of haustorium. In addition, upregulation of genes that encode cell wall-modifying enzymes was detected in the transcriptome of host-parasite interface in the model Triphysaria versicolor, using laser microdissection. These aforementioned results suggest that parasitic plants facilitate cell–cell adhesion at the interface between the haustorium and host through activation of cell wall-modification enzymes.

In this study, we addressed molecular commonality between Pr. japonicum and Arabidopsis by comparing tissue adhesion events between Phleum pratense and Arabidopsis. Although these events occur in different organs, we expected that key common components for tissue adhesion would be found by comparative transcriptomic analyses. In addition, we further compared these datasets with that of interfamilial graft of Nicotiana benthamiana, which is able to adhere cells with those of plant species from diverse families in grafting. We identified nine genes that were commonly upregulated in P. japonicum haustorium, Arabidopsis, and N. benthamiana/Arabidopsis grafting sites. Among them, we identified a gene encoding β-1,4-glucanase as an important factor in plant parasitism.

**Results**

**Tissue adhesion between P. japonicum and Arabidopsis in parasitism and grafting.** A facultative parasitic plant, P. japonicum, has been studied previously as a model root parasite that can parasitize Arabidopsis. The ability to transport materials from Arabidopsis to P. japonicum can be visualized using a symplasmic tracer dye, carboxyfluorescein (CF). At the parasitization site in the root, a xylem bridge is formed in the haustorium, by which the P. japonicum tissues invade the host tissues. We observed the interface of the P. japonicum haustorium and Arabidopsis root tissues using transmission electron microscopy (Fig. 1e–i). The cells at the tip of the penetrating haustoria adhered closely to the opposing Arabidopsis cells where thin cell walls were observed. Serial sections revealed a decrease in cell wall thickness at the interface between P. japonicum and Arabidopsis tissues (Fig. 1f–i), which indicated that cell wall digestion occurred at the interface.

We observed similar thin cell walls at graft boundary between Arabidopsis and Nicotiana species, which exhibit a capability to adhere their tissue across interfamilial species. Therefore, we hypothesized that the parasitic plant may also have a wide tissue adhesion capability in artificial grafting. To test this hypothesis, we grafted a stem of P. japonicum (as the scion) onto the Arabidopsis inflorescence stem. Cells proliferated on the cut surface of the scion, similar to haustorial tissues. The P. japonicum scion was able to establish a graft union with the Arabidopsis scion and remained viable for 1 month after grafting. Given that parasitic Orobanchaceae species have a diverse host range among angiosperms, we further tested graft combinations using nine species from seven orders of angiosperms. The grafting capability of P. japonicum as the scion using these interfamilial species as the stock was clearly observed, except for two Fabaceae species (Fig. 2d–f and Supplementary Data 1). Reciprocally, P. japonicum was able to be used as the stock plant for certain plant species (Fig. 2g and Supplementary Data 1). In contrast, when Lindenbergia philippinensis, which has no parasitic ability among Orobanchaceae, was grafted onto Arabidopsis, viability of the L. philippinensis scion was extremely limited. When we observed cross-sections of the graft junction of P. japonicum/Arabidopsis (scion/stock), xylem continuity, and apoplastic dye transport were observed (Fig. 2h, i). Importantly, establishment of the
symplast between *P. japonicum* and *Arabidopsis* was confirmed by using the CF dye (Fig. 2j). In summary, these results showed that the root parasite *P. japonicum* is able to achieve tissue adhesion and vasculature connection with members of diverse plant families in both parasitism and grafting.

**Transcriptome analyses of parasitism and grafting.** To investigate molecular events involved in cell–cell adhesion between *P. japonicum* and the host plant, we analyzed the transcriptome for *P. japonicum*–*Arabidopsis* parasitism and *P. japonicum*/*Arabidopsis* grafting. For this purpose, taking into account the periods during which parasitism and grafting between distantly related plants are established, sequential samples of haustorial infection sites at the roots from 1 to 7 days post infection (DPI) and of plants are established, sequential samples of haustorial infection during which parasitism and grafting between distantly related plant families in both parasitism and grafting.

Methods

**Fig. 1 Parasitism of *P. japonicum*. a, b** Parasitism between the roots of *P. japonicum* and *Arabidopsis*. The *P. japonicum* root parasitized the *Arabidopsis* root (insets). Transport of a symplasmic tracer dye, carboxyfluorescein (CF, colored in green), showed establishment of a symplasmic connection between the plants; light micrograph (a) and fluorescence image (b). Arrows indicate the site where CF dye was applied and the direction of transport. **c, d** Site where *P. japonicum* parasitized the *Arabidopsis* root. **c** Phloroglucinol staining showing xylem bridge formation (XB). **d** Cross-section of the parasitization site. The *P. japonicum* tissue invaded the *Arabidopsis* root tissues. Dashed line indicates the interface of parasitism. **e** Transmission electron micrograph of the interface between *P. japonicum* (pink) and *Arabidopsis* (blue). Partial tissue adhesion was observed at the interface. The dashed rectangle indicates the area of (f–i). **f–i** Serial sections at the interface between *P. japonicum* and *Arabidopsis* cells. The cell wall was partially digested. Pj *P. japonicum*, At *Arabidopsis*. Scale bars, 5 mm (a, b), 100 μm (c, d), 10 μm (e), and 2 μm (f–i).
parasitization of the host root, tissue adhesion between *P. japonicum* and the host occurred around 1–2 DPI, and then a xylem bridge connecting a *P. japonicum* root vessel and a host vessel was formed at 3 DPI (Fig. 4a). In contrast, histological observation showed that tissue adhesion between the scion and stock during grafting occurred about 3 DAG (Fig. 4b). We focused three clusters with distinct expression patterns (Fig. 4a, b). The first one includes genes upregulated during the tissue adhesion stage starting about 1 DPI or 1 DAG (Node 09 for parasitism, Node 10 for grafting). The second one contains genes upregulated along the time (Node 05 for parasitism and Node 08 for grafting). The third one includes genes peaked around 2 DPI or 3 DAG (Node 08 for parasitism, Node 10 for grafting). The second one contains genes upregulated along the time (Node 05 for parasitism and Node 08 for grafting). The third one includes genes peaked around 2 DPI or 3 DAG (Node 08 for parasitism, Node 10 for grafting). The second one contains genes upregulated along the time (Node 05 for parasitism and Node 08 for grafting). The third one includes genes peaked around 2 DPI or 3 DAG (Node 08 for parasitism, Node 10 for grafting). Genes encoding glycosyl hydrolases were associated with cell division, such as cyclin D, and cell wall-related genes, such as glycosyl hydrolase (Fig. 5). One of the identified glycosyl hydrolases belongs to the *Glycosyl hydrolase 9B* (*GH9B*) family, which includes genes encoding β-1,4-glucanases in plants.28,29 Interestingly, among the *GH9B* family, a member of *GH9B3* clade was recently shown to be crucial for cell–cell adhesion in *Nicotiana* interfamily grafting.21

**PjGH9B3 is essential for *P. japonicum* parasitism.** As cell walls locating at the interface between *P. japonicum* and *Arabidopsis* were partially digested (Fig. 1e, f), we decided to analyze function of the *GH9B3* in parasitism. We reconstructed a phylogenetic tree for the *GH9B* gene family for *Arabidopsis*, *P. japonicum*, and *S. hermonthica*, as well as *L. philippensis*, a nonparasitic Orobancheae species.30 (Fig. 6). In the phylogenetic tree, five and four genes from *P. japonicum* and *S. hermonthica* are found in the *GH9B3* clade, respectively, while only two and one *GH9B3* genes are present in *Arabidopsis* and *L. philippensis*, respectively.
**Fig. 3** Transcriptomic analysis of parasitism and grafting between *P. japonicum* and *Arabidopsis*. **a** Transcriptomic analysis was performed using RNA samples of the *P. japonicum* infected site and the *P. japonicum* graft site. For parasitism, total RNA was extracted before and 1, 2, 3, 5, and 7 days post infection (DPI). For grafting, total RNA was extracted before and 1, 2, 3, 4, 5, 6, 7, and 14 days after grafting (DAG) (with four biological replicates for each time point). Principal component analysis was performed from the obtained expression profile. Triangles and circles represent parasitism and grafting, respectively. **Table:** principal component. **b** Hierarchical clustering using Euclidean distance and Ward’s minimum variance method over ratio of RNA-seq data from five time points for *P. japonicum–Arabidopsis* parasitism and *P. japonicum/Arabidopsis* grafting against intact plants. Genes for which association with parasitism and grafting has been reported in previous studies are marked. Using the cDNA sequence of *Arabidopsis* as queries, tblastx was used to determine the most closely related homologs of *P. japonicum*. **c** Plots of the gene expression levels marked in **b**. Red background represents genes that behaved similarly in parasitism and grafting, and blue background indicates genes that behaved differently.

Moreover, the tendency that the number of genes belonging to the GH9B3 clade were increased only in the parasitic plant was also found in the phylogenetic trees for *Striga asiatica*, another species of the genus *Striga*, and *Erythranthe guttata* (*Mimusulus guttatus*), a nonparasitic plant of the Lamiaceae (Supplementary Fig. 2a, b). The LpHiGnB1_1726, a member of the *L. philippensis* GH9B3 clade was upregulated at 1 DAG, but such upregulation was observed even in incompatible graft combinations using other plant species; soybean (*Glycine max*), morning glory (*Ipomoea nil*), and *Arabidopsis*11. However, expression did not continue to increase subsequently, as this is often the case for incompatible graft combinations11 (Fig. 6b). By contrast, *Pjv1_G00028629*, the most similar *P. japonicum* homolog of *NbGH9B3* gene that was associated with grafting, was upregulated at 1 DAG and gradually increased until 7 DAG in grafting, as well as 7 DPI in parasitism (Fig. 6b). Similarly, in *S. hermonthica*, the corresponding homolog *Sh14Contig_25152* was upregulated at 1 DPI with a peak at 3 DPI16 (Fig. 6b). Some of the other homologous genes of the GH9B3 clade were also upregulated in parasitism, but not for those of the other GH9 clades (Supplementary Fig. 3). These data suggest that upregulation of GH9B3 homologs in parasitic plants at the site of infection and interfamily grafting is conserved in parasitic Orobanchaceae plants.

*Pjv1_G00028629* is upregulated at the early phase of infection and is phylogenetically closest to the GH9B3 genes of *Nicotiana* and *Arabidopsis*. Alignment analysis of amino acid sequences shows that four genes including *Pjv1_G00028629* conserve a catalytic domain and O-glycosylation sites (Supplementary Fig. 4). Therefore, we investigated the function of *Pjv1_G00028629*, called *PjGH9B3*. First, temporal and spatial expression patterns of *PjGH9B3* were examined using a promoter-Venus construct. *PjGH9B3* promoter activity was detected at the cell periphery of the haustorium attaching to the host at 1–2 DPI (Fig. 7a, b), while the signal was later shifted to the inside of haustorium at 3–4 DPI (Fig. 7c, d). These expression patterns indicated that *PjGH9B3* functions at the interface with the host root tissue at the early adhesion stage and xylem formation at the later stage. *PjGH9B3*-knockdown experiments by RNA interference (RNAi) system revealed that *PjGH9B3*-knockdown did not affect haustorium emergence but resulted in significantly fewer successful xylem connections with host, compared with the control (Fig. 7e–i). Reduction of *PjGH9B3* expression was confirmed in hairy roots using quantitative reverse-transcription PCR (RT-qPCR) (Fig. 7f). These data indicate that *PjGH9B3* positively regulates infection processes in *P. japonicum*. 

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**Fig. 4** Comparison of self-organizing map (SOM) clusters associated with the tissue adhesion stage during parasitism and grafting. **a, b** Tissue sections of the parasitized site of *P. japonicum* 12 h post infection (HPI), 1, 2, and 3 DPI (a) and the graft junction between *P. japonicum* (*Pj*) and *Arabidopsis* (*At*) 1, 3, and 5 DAG (b). Fluorescence images of the graft junction are also shown where *P. japonicum* was grafted onto *Arabidopsis* harboring *RPS5a::LTI6b-tdTomato* (b). Green indicates the cell wall, magenta indicates tdTomato fluorescence. Xy, xylem; XB, xylem bridge. SOM clusters with similar patterns in parasitism (a) and grafting (b) are shown. **c** Enriched gene ontology (GO) terms common to parasitism and grafting are listed for the clusters. Nodes 5, 8, and 9 were merged from the SOM cluster of the *P. japonicum* parasitism transcriptome, and nodes 8, 10, and 11 were merged from the SOM cluster of the *P. japonicum* grafting transcriptome. A portion of the GO terms categorized as "biological processes", "cellular components", and "molecular functions" are shown. GO terms potentially associated with "cell division" and "cell wall modification" are marked in blue and red, respectively. Scale bars, 50 µm (a), and 100 µm (b).
**Discussion**

Parasitic invasion by haustorium is established through the disruption of host cell wall barriers following attachment of haustorium to the host tissues by haustorial hairs. Similar modification of the cell wall is also observed in the graft, which is an artificial tissue connection. In general, grafting is successful between closely related plants, such as members of the same species, genus, and family, but not between genetically distant plants, such as members of different families, with a few exceptions, such as Nicotiana, which we previously reported. Since the adhesion of plant tissues of different families also occurred in parasitism, we expected that there might be a commonality between the capability of these parasitic plants to parasitize diverse hosts and their capability to implement heterografting. In support of our assumption, inter-family grafting of *P. japonicum* showed its compatibility with multiple autotrophic plants (Fig. 2c–g and Supplementary Data 1). Because previous studies in *N. benthamiana* displayed that a higher success rate of grafting could be achieved when it was used as a scion rather than a stock, we grafted the *P. japonicum* plant mainly as a scion in this study. Remarkably, grafting capability of *P. japonicum* was also observed when it was grafted as a stock onto several plant species, indicating the fundamentally high grafting
Secondary cell walls are formed in mature cells and are composed of pectic polysaccharides, and hemicelluloses such as xyloglucans. Growing tissues and are composed predominantly of cellulose, and the resulting diverse cell wall families and character, such as cell proliferation and differentiation abilities, or their combinations. Another observation was that parasitic plants may have acquired a mechanism to reconstruct the clade of parasitism (Fig. 6b). Gene duplication is involved in the evolution of parasitism. A previous study showed that duplication events occurred in more than half of the parasitic genes of Orobanchaceae species, which comprise a large number of genes annotated with GO terms associated with cell wall-modifying enzymes and peptidase activity. Duplicated genes are potential drivers of functional innovation and adaptive evolution. Notably, the number of GH9B3 and GH9B8 genes increases in Orobanchaceae parasites (P. japonicum, S. asiatica, and S. hermonthica) but not in non-parasites (E. guttata and L. philippensis), suggesting that these genes may have been co-opted to plant parasitism in the Orobanchaceae (Fig. 6a and Supplementary Fig. 2). In contrast, no notable expansion was observed for other clades of the GH9 family (Fig. 6a and Supplementary Fig. 2). Gene duplication observed in the KA12 strigolactone receptor family in S. hermonthica is also considered to be a driver for successful parasitism. The number of GH9B3 clade genes in P. japonicum is not notably higher compared with the KA12 duplication event in S. hermonthica. In the case of GH9B3 genes, four out of five homologous genes in P. japonicum were predicted to encode functional proteins in our alignment sequence analysis (Supplementary Fig. 4), but their expression patterns were temporally varied (Figs. 6b, 7a–d, and Supplementary Fig. 3a). Taken together, we assume that duplication of GH9B3 and the resulting diversification in spatio-temporal expression are both important factors in the evolution of parasitism.

We narrowed the genes associated with tissue adhesion among distant species (Figs. 4 and 5) and identified a role in parasitism for one of candidate genes using P. japonicum system (Figs. 6 and 7).
Additional important components are likely to facilitate cell wall digestion and accomplish tissue adhesion. The dataset accumulated in the present study provides a foundation to identify such components involved in parasitic infection and/or grafting. *P. japonicum* is a useful model system because the seeds can germinate in the absence of a host plant, obtain transformants from hairy roots, parasitize the host, and can be grafted to the host plant species**32,23,40** (Figs. 1 and 2). Improved knowledge of the mechanisms responsible for parasitism could be applicable to suppress yield losses in crop cultivation caused by parasitic plants. The present study may provide an option, which might be effectively applied to hemipterans, to decrease parasitization by parasitic plants after germination by inhibiting the activities of secreted endo-β-1,4-hexanases. Several mono- and polysaccharides are reported to be inhibitors of cellulases**21,23,40**. Hence, a knowledge-based defense approach would further enhance crop security.

**Methods**

**Plant materials.** For the grafting experiments, *P. japonicum* and *Arabidopsis thaliana* ecotype Columbia (Col-0) seeds were directly surface-sterilized on soil. Plants were germinated on Murashige and Skoog continuous light plates. The same indeterminate cell wall digestion experiment was as described previously**22,23. Briefly, *Arabidopsis* seeds were surface-sterilized with 70% ethanol for 10 min, washed three times with sterile water, incubated at 4°C for 2 days, and sown on half-strength Murashige and Skoog (1/2 MS) medium (0.8% agar, 1% sucrose). The agar plates were incubated at 4°C in the dark overnight, then rinsed with 10% (v/v) commercial bleach solution (Kao, Tokyo, Japan) for 5 min, rinsed three times with sterilized water, and sown on 1/2 MS agar medium (0.8% agar, 1% sucrose). The agar plates were incubated at 4°C in the dark overnight, then incubated at 25°C under LD. Plants for the infection assay and the transformation experiment were cultured vertically and horizontally, respectively.

**Grafting.** For grafting of *P. japonicum*/*Arabidopsis*, 1–2-month-old *P. japonicum* and 1-month-old *Arabidopsis* plants were used. Wedge grafting was performed on the epidermis, when the petiole, or peduncle (preparation, the same organs) were cut with a 2.3–2 cm slit on the top. For scion preparation, the stem (around 7–10 cm from the tip of the stem) was cut and trimmed into a V-shape. The scion was inserted into the slit of the stock and wrapped with paraffin to maintain close contact. A plastic bar was set along the stock and the scion to support the scion and the graft. The entire scion was covered with a plastic bag, which had been sprayed inside with water beforehand. Grafted plants were grown for 7 days in an incubator at 27°C under continuous light (~30 µmol m⁻² s⁻¹). After this period, the plastic bags were partly opened by cutting the bags and making holes for acclimation. The next day, the plastic bags were removed and the grafted plants were governed to a plant growth chamber at 22°C under continuous light conditions (~80 µmol m⁻² s⁻¹). All other plant materials for stem grafting used in this study are listed in Supplementary Data 1.

**Microscopy.** All the chemicals for staining were purchased from Sigma-Aldrich Co., Tokyo, Japan unless otherwise stated. Preparation of resin sections and observation by a transmission electron microscope were performed as previously described**21,22. In this study, we showed other sections of the same grafted plant sample prepared in previous study**21. To capture images of infections tissues or hand-cut grafted regions, a stereo-microscope (SZ61, Olympus, Tokyo, Japan) equipped with an on-axis zoom microscope (Axio Zoom.V16, Zeiss, Göttingen, Germany) was used to observe xylem tissues, phloroglucinol staining (Wieuner reaction) was performed by applying 18 µl of 1% phloroglucinol in 70% ethanol followed by the addition of 18 µl of 10% hydroxylamine. From these observations, we determined apoplastic transport, the stems of *Arabidopsis* stocks were cut and the cut edge was soaked in 0.5% toluidine blue solution for 4 h to overnight. To determine symplastic transport, cut leaves of the *Arabidopsis* host or stock were treated with 0.1% (w/v) CF diacetate. The CF fluorescence images were acquired by a fluorescence imaging stereomicroscope or observing emissions in the 490–530 nm range with excitation at 488 nm with a confocal laser scanning microscopy (LSM780, Zeiss). To examine graft junctions and visualize tissue adhesion, grafting was performed between a *P. japonicum* scion and a transgenic *Arabidopsis* stock, RPPSA:A:1106:ttUTomato, which ubiquitously expresses a plasma membrane-localized fluorescent protein**23. The probe was mixed thoroughly in the solution at a concentration of ~10–15 mm of graft junction or stem tissue at a similar location were sampled at the represented time points. For parasitism and grafting samples between *P. japonicum* and *Arabidopsis*, the sequence reads were mapped on the genome assembly using TopHat version 2.1.0 with default parameters (http://ccb.jhu.edu/software/tophat/). Uniquely mapped reads are counted using HTSeq (https://htseq.readthedocs.io/en/master/), and high homology genes that show more than ten total reads per million fragments mapped, were estimated using Cufflinks version 2.1.1 (http://cole-trapnell-lab.github.io/ cufflinks/). The reference sequence used for mapping and the annotation file used were as follows: *P. japonicum* PjScaffold_v1; *N. benthamiana* draft genome sequence v1.0, https://science.scripps.edu/scripsexploits/resources/nicotiana-benthamiana/; *A. thaliana* TAIR10 genome release, https://www.arabidopsis.org and *L. philippensis* LiPghBn1, http://pppg.huck.psu.edu. GO enrichment analysis was performed with DAVID (https://david.ncifcrf.gov) using *Arabidopsis* gene IDs. Transcriptome data of parasitism and grafting were used for PCA to compare the differences between samples. The Python version 3.7.4 and its libraries (including NumPy 1.17.2, Pandas 0.23.1, SciPy 1.1.3), seaborn (0.9.0), and scikit-learn (0.21.3) were used for PCA and hierarchical cluster classification. For SOM clustering, the parasitism was classified into nine clusters and the grafting was classified into 12 clusters. After clusters with similar patterns were paired, three clusters with increased expression during cell adhesion of both the parasitism and the grafting, and the clusters located before and after those were presented.

**Plasmid construction.** We used Golden Gate modular cloning to construct a vector to examine the expression pattern of PfGH9B3 during infection**39. The PfGH9B3 promoter region (1899 bp upstream of the ATG start codon) was cloned into the vector pAGM1311 as two fragments and then combined into the vector pCH41295 to generate the promoter module. This module was assembled into the vector pCH4775 containing 3xVenus-NLS and AHsp1B terminator**22, then subsequently further assembled into pAGM1311 with pJACT::3xmCherry-NLS**22. For RNAi experiments, we used the pHG8-YFP vector**42. Target sequences, from 1175 to 1444 in coding sequence, were PCR-amplified from *P. japonicum* genomic DNA and cloned into the pENTR vector (Thermo Fisher Scientific, Waltham, MA, USA), then transferred into the pHG8-YFP vector by the Gateway reaction using LR Clonase II Plus enzyme (Thermo Fisher Scientific). All primers used in this paper are listed in Supplementary Data 11.

**P. japonicum transformation.** *P. japonicum* transformation was performed as previously described**24. Six-day-old *P. japonicum* seedlings were washed with water and then vacuumed for 10 s and 5 min, respectively, in an aqueous suspension of Agrobacterium rhizogenes strain AR1193. After incubation in freshly prepared Gamborg’s B5 medium (0.8% agar, 1% sucrose, 450 µg/mL acetosyringone) at 22°C in the dark for 2 days, seedlings were incubated in Gamborg’s B5 medium (0.8% agar, 1% sucrose, 300 µg/mL cefotaxime) at 25°C under LD.

**Parasitism assay and RT-qPCR.** Ten-day-old *P. japonicum* seedlings were transferred from 1/2 MS medium agar plates to 0.7% agar plates and incubated at 25°C under LD for 2 days. Seven-day-old *Arabidopsis* seedlings were placed next to the *P. japonicum* root tips, which grow in the same plate. To determine apoplastic transport, the stems of *Arabidopsis* stocks were cut and the cut edge was soaked in 0.5% toluidine blue solution for 4 h to overnight. To determine symplastic transport, cut leaves of the *Arabidopsis* host or stock were treated with 0.1% (w/v) CF diacetate. The CF fluorescence images were acquired by a fluorescence imaging stereomicroscope or observing emissions in the 490–530 nm range with excitation at 488 nm with a confocal laser scanning microscopy (LSM780, Zeiss). To examine graft junctions and visualize tissue adhesion, grafting was performed between a *P. japonicum* scion and a transgenic *Arabidopsis* stock, RPPSA:A:1106:ttUTomato, which ubiquitously expresses a plasma membrane-localized fluorescent protein**23. Hand-cut cross-sections of the grafted stem regions were stained with 0.001% calcofluor white, which stains cellulose in plant cell walls. The fluorescence of tdTomato or calcofluor white was detected using a confocal laser scanning microscope (LSM780, Zeiss). A 555 nm laser and collecting emission spectrum of 560–660 nm were used for tdTomato and a 405 nm excitation laser and collecting emission spectrum of 420–475 nm were used for calcofluor white. The GFP or mCherry fluorescence images were acquired as described previously**22.
Statistical and reproducibility. The details about statistics used in data analyses performed in this study are described in the respective sections of results and methods. For transcriptome analysis, we used four independent replicates of grafted or infected *P. japonicum* and *Arabidopsis*, including ten independent plants in each experiment. For Phenotype-Seq knockdown experiments, the number of biological replicates and plant samples are given in figure legend.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**
The RNA-Seq data are available from the DNA Data Bank of Japan (DDBJ; http://www.ddbj.nig.ac.jp/) under the accession number, DRA010010. All the source data underlying the graphs and charts are presented in Supplementary Data 2, 3, 12 and 13.

**Code availability**
The details of publicly available program used in this study are described in the section of “Methods”. No custom code or mathematical algorithm that is deemed central to the conclusions were used in this study.

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Author contributions
M.N., Y.I., K.S. and K.K. conceived the research and designed the experiments. M.N., K.O., and Y.S. performed the grafting experiments and morphological analysis. Y.I., T.S., S.C., and K.K. performed the RNA-Seq analysis. Y.I. performed the SOM clustering analysis. K.K. analyzed the transcriptome data. T.W. and S.O. performed functional analysis of the candidate genes. M.N. and K.S. supervised the experiments. K.K., T.W., K.S. and M.N. wrote the manuscript.

Competing interests
The authors declare no competing interests.

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