An *Arabidopsis* receptor-like kinase mediates competitive plant-plant interactions

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Summary

While competition among plant species is recognized as a major factor affecting crop yield and plant community dynamics, the genetic and molecular mechanisms underlying natural variation of such biotic interactions remain poorly characterized. Here, we report the cloning of a Quantitative Trait Locus previously detected in a Genome-Wide Association Study investigating the competitive response of the tale cress *Arabidopsis thaliana* to the presence of the annual bluegrass weed species *Poa annua*. Using mutant and complementation strategies, we identified *ESCAPE 1* (*ESC1*) as the gene responsible for the natural variation of an escape strategy of *A. thaliana* in response to the presence of *P. annua*. *ESC1* encodes a proline-rich, extensin-like receptor kinase, also known as PERK13 (AT1G70460). An RNA-seq experiment revealed that PERK13 functions through different pathways in leaves and roots involving genes associated with responses to biotic and abiotic stresses. Using these RNA-seq together with yeast two-hybrid (Y2H) data, protein-protein interaction network reconstruction revealed two distinct decentralized protein networks in leaves and roots. These findings support the notion of an active response mechanism involved in neighbor detection. The functional validation of *ESC-1* underlying natural variation in response to competition opens new avenues for a better understanding of the molecular dialogue involved in plant-plant interactions.

Keywords: *Arabidopsis thaliana*, plant-plant interactions, receptor-like kinase, competitive response, natural variation.

Significance statement

Although competition among plant species significantly impacts the performance of crops and wild plants, the genetic and molecular bases of natural variation in these interactions remain largely unknown. In this study, we cloned a receptor-like kinase involved in the response of *Arabidopsis thaliana* to various neighboring species. Through an integrated approach combining genetics, transcriptomics, and systems biology, we uncovered two distinct genetic pathways that support active mechanisms for neighbor detection in *A. thaliana*.

Introduction

In natural environments, the structure, diversity, and dynamics of plant communities are largely shaped by competitive interactions among species (Tilman 1985; Goldberg and Barton 1992; Martorell and Freckleton 2014). Similarly, in the absence of pesticides, competitive interactions between weeds and crop species are the primary biotic factor limiting crop biomass and grain yield, accounting for approximately 36% of losses, compared to about 18% caused by animal pests and 16% by pathogens (Oerke 2006; Neve et al. 2009). Despite the importance of interspecific competition in the functioning of natural plant communities and crop performance, our understanding of the genetic and molecular bases underlying natural variation in interspecific competition is limited compared to other types of biotic interactions such as plant-pathogen interactions (Roux and Bergelson 2016; Subrahmaniam et al. 2018; Becker et al. 2023).

To our knowledge, only four traditional Quantitative Trait Loci (QTL) mapping studies (using F2 or Recombinant Inbred Lines mapping populations) and three Genome-Wide Association mapping Studies (GWAS), reported the genetic architecture underlying the competitive response of a focal species to the presence of neighboring species (Coleman et al. 2001; Moncada et al. 2001; Granberry et al. 2016; Asif et al. 2015; Baron et al. 2015; Frachon et al. 2017; Libourel et al. 2021). Across these studies, the genetic architecture was consistently polygenic, ranging from the identification of a few medium-effect QTLs to dozens of small-effect QTLs (Subrahmaniam et al. 2018; Sato and Wuest 2025). In addition, the genetic architecture was strongly influenced by the identity of neighboring species, the diversity of surrounding species, and the composition of the plant assemblage (Libourel et al. 2021). In the three GWAS, all considering *Arabidopsis thaliana* as the focal species (Baron et al. 2015; Frachon et al. 2017; Libourel et al. 2021; Walsche et al. 2025), the fine mapping of genomic

regions associated with natural variation in the response to the presence of neighboring species revealed numerous candidate genes related to key plant functions, including cell wall modification, signaling and transport. Interestingly, these categories were rarely identified in experiments conducted under artificial conditions simulating plant-plant interactions, such as shading (Subrahmaniam et al. 2018). Furthermore, QTL cloning of genes associated with natural variation in interspecific competition has yet to be conducted, leaving the molecular mechanisms underlying this aspect of plant biotic interactions largely unexplored.

In this study, we identified and conducted a functional analysis of a gene underlying a QTL involved in the response to competition in *A. thaliana*. We used the data of a previous GWAS involving 91 accessions of *A. thaliana* from the highly genetically polymorphic French local mapping population TOU-A, grown either in the absence or in the presence of the bluegrass *Poa annua* (**Figure 1A**; Libourel et al. 2021). *P. annua* is a common weed in cultivated fields (Li et al. 2009) and one of the primary grasses co-occurring with *A. thaliana* in natural plant communities and permanent meadows (Frachon et al. 2017, 2019). We targeted a QTL that explains approximately ~15% of the genetic variation in both plant height from the soil to the first flower on the main stem and the height-to-diameter (HD) ratio, defined as the ratio between plant height and rosette diameter (**Figure 1B**; Libourel et al. 2021). High and low HD ratios have been shown to correspond to escape and aggressive strategies of *A. thaliana* in response to competition, respectively (Baron et al. 2015). Moreover, natural variation in the HD ratio in *A. thaliana* is under selection in the TOU-A population, which inhabits a highly competitive environment (Frachon et al. 2017). We therefore aimed to clone the causal gene *ESC1* underlying the QTL associated with the natural variation in the HD ratio. Among the genes located in this region, we demonstrated that *ESC1* corresponds to *PERK13* and mediates the escape strategy of *A. thaliana* in response to the presence of *P. annua*. In addition, through transcriptomics, yeast two-hybrid screening, and network reconstruction, we demonstrated that

ESC1/PERK13-mediated pathways involve genes associated with both biotic and abiotic stress responses. Our findings suggest that plant responses to competition extend beyond competition for resources and engage molecular mechanisms similar to those involved in plant immune responses.

Results

ESC1* corresponds to *PERK13* and mediates the competitive response to *Poa annua

To investigate the molecular mechanisms involved in the natural variation of *A. thaliana* competitive response to *P. annua* (**Figure 1A** and **1B**), we aimed at cloning the gene underlying a QTL explaining ~15% of the genetic variation in the HD ratio (Libourel et al. 2021), hereafter called *ESCAPE1* (*ESC1*). A close-up revealed that this QTL, located at the end of chromosome 1, corresponds to a neat association peak spanning ~20kb (**Figure 1C**). This short genomic region includes four genes, namely *AT1G70440* (*SIMILAR TO RCD ONE3*), *AT1G70450*, *AT1G70460* (*PROLINE RICH, EXTENSIN-LIKE RECEPTOR KINASE 13 - PERK13*, also named *ROOT HAIR SPECIFIC 10 - RHS10*), and *AT1G70470*.

To identify the gene underlying the natural genetic variation in the HD ratio in response to *P. annua*, we measured the phenotypic response to the presence of *P. annua* of eight T-DNA mutant lines corresponding to the genes located within the 20kb region of the QTL (**Figure 1C** and **Table S1**). The wild-type Col-0 accession exhibited a significant increase (+40%) in the HD ratio in response to the presence of *P. annua* compared to the control condition (**Figure 1D**). Seven T-DNA mutants exhibited a response similar to Col-0, although the increase in HD ratio observed in mutant *#6* was not statistically significant (**Figures 1D, Table S2**). On the other hand, no increase in the HD ratio was observed for the loss-of-function mutant #7 (hereafter named *perk13.1*), suggesting that *PERK13* plays a major role in the competitive response to *P. annua*. In line, a genotype overexpressing *PERK13* in root hairs, *PERK13*Ox

(Hwang et al. 2016), exhibited a constitutive higher HD ratio than Col-0 in the absence of *P. annua* as well as an increase in the HD ratio in response to the presence of *P. annua*.

A functional complementation of the *perk13.1* mutant with a ~5.6kb genomic region including the Col-0 *PERK13* allele in three independent complemented lines (*perk13.1*|*PERK13*-Col-0) led to a complete restoration of the response to wild-type level (**Figures 2B** and **2C**, **Table S3**). Together, these results demonstrate that *ESC1*/*PERK13* plays a significant role in the competitive response of *A. thaliana* to the presence of *P. annua* by mediating an above-ground escape strategy.

Then, we explored the specificity of *ESC1/PERK13* in the competitive response by growing Col-0, *perk13.1,* and *PERK13*Ox lines in the presence of *P. annua*, as well as four other grass species (including two wild species and two crops), and two herb species commonly associated with *A. thaliana* in natural plant communities (**Figure 1E** and **Table S4)**. We observed similar phenotypic responses between Col-0 and *perk13.1* across most herb and grass species. However, in the presence of the common wheat *Triticum aestivum*, *perk13.1* displayed a lower HD ratio than Col-0, while *PERK13*Ox showed a higher HD ratio, therefore mirroring the response observed with *P. annua*. These results indicate that the role of *PERK13* in the competitive response of *A. thaliana* depends on the identity of the neighboring species.

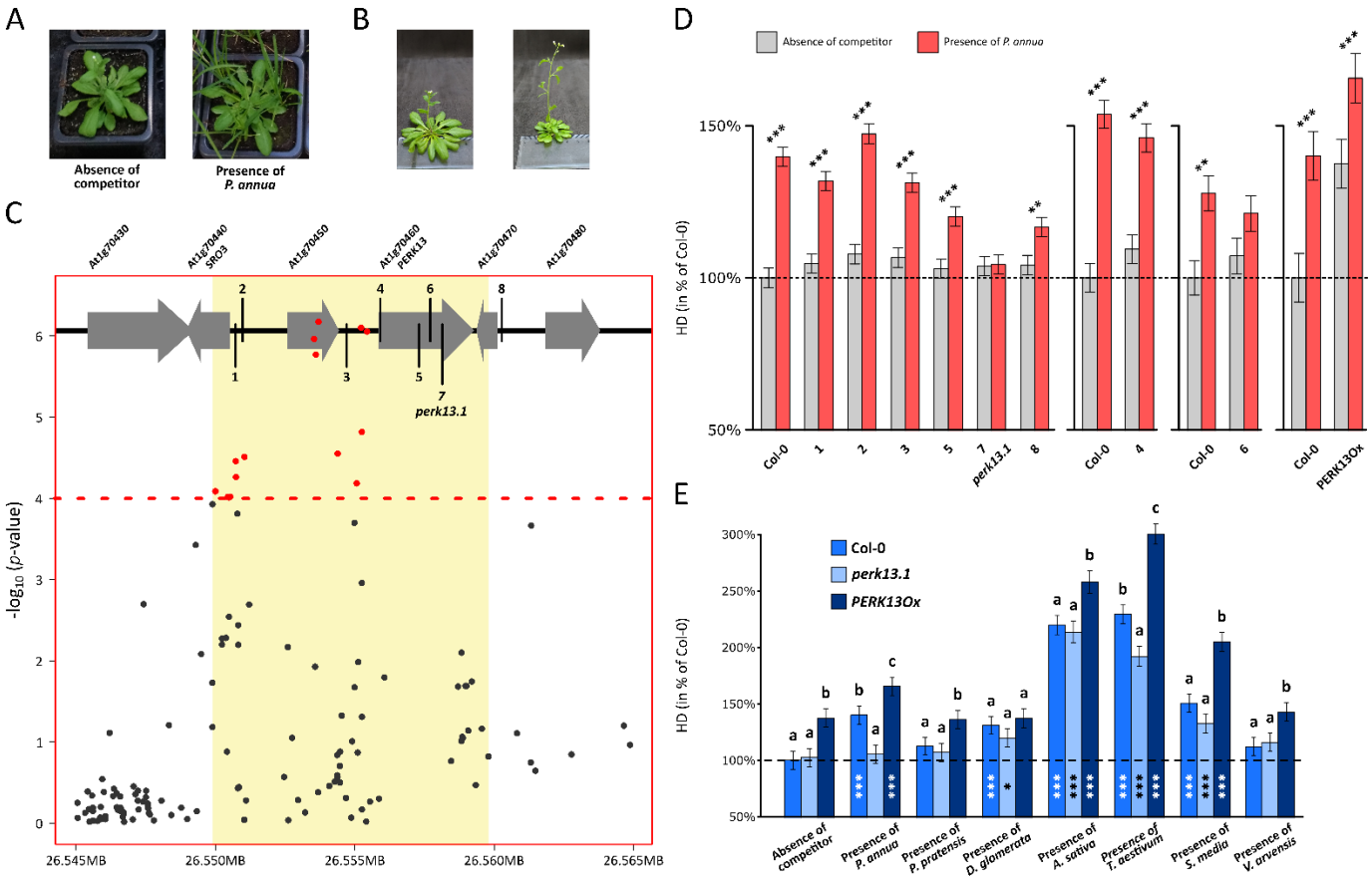


Figure 1. *ESC1/PERK13* contributes to the escape strategy of *A. thaliana* in response *to P. annua.* (A) *A. thaliana* plants growing in the absence or the presence of *P. annua.* (B) Natural variation of HD ratio illustrated by two accessions with contrasted phenotype. (C) Close-up view of the association peak identified for HD ratio in response to *P. annua* in Libourel et al., 2021, with a schematic representation of the genes located in the interval of the QTL identified. The numbered vertical bars represent the position of the selected mutant lines. The red dots indicate the most significant associated SNPs ($-\log_{10}(p\text{-value}) > 4$). The yellow rectangle indicates the confidence interval detected by a local score approach (Bonhomme et al., 2019). (D) Bar plots of the Least Square means (LSmeans) of the HD ratio from the tested *A. thaliana* genotypes in the absence (grey bars) or the presence (red bars) of *P. annua*. The values are expressed as a percentage of the HD ratio measured on Col-0 in the absence of *P. annua,* with numbers corresponding to each mutant line. Phenotypes were obtained from at least three independent experiments. FDR corrected *p*-values: $*0.05 > P > 0.01$, $**0.01 > P > 0.001$, $*** P < 0.001$, absence of symbols: non-significant. (E) Bar plots illustrate the specificity of *ESC1/PERK13* towards other plant species besides *P. annua.* LSmeans of the HD ratio in the absence and presence of seven species (*P. annua*, *Poa pratensis*, *Dactylis glomerata*, *Avena sativa*, *Triticum aestivum*, *Stellaria media*, *Veronica Arvensis*) expressed as a percentage of the HD ratio measured on Col-0 in absence of competitor. FDR corrected *p*-values for each genetic line between each treatment of interspecific competition and absence of competitor: $*0.05 > P > 0.01$, $**0.01 > P > 0.001$, $*** P < 0.001$, absence of symbols: non-significant. For each treatment, different letters indicate different groups according to the genetic lines after a FDR correction.

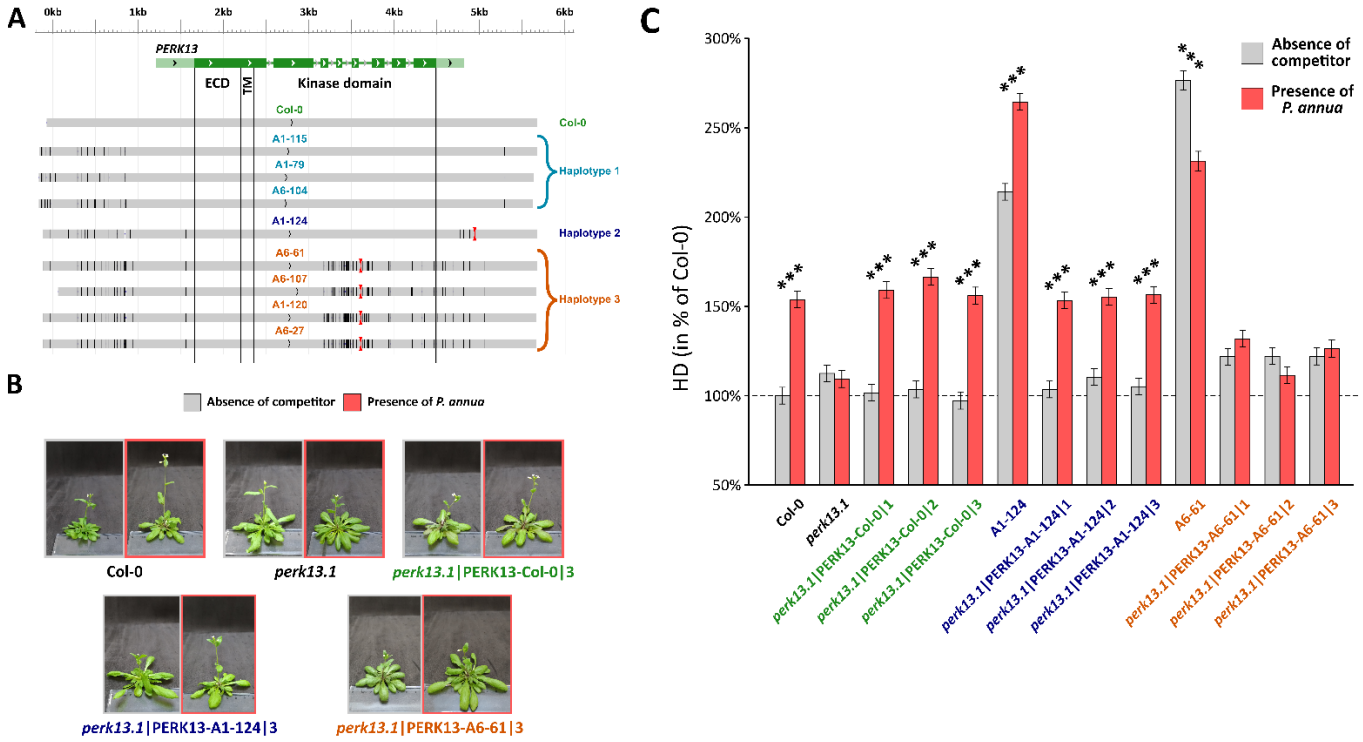


Figure 2. *ESC1/PERK13* genetic diversity controls the natural variation of *A. thaliana* response to the presence of *P. annua*. (A) Sequence diversity observed in a ~5.6kb region centered on *PERK13* in Col-0 and eight accessions from the TOU-A population. Black vertical lines indicate mismatches, and white vertical lines indicate deletions. Insertions are represented by red hourglasses. (B) Illustration of the HD ratio with representative pictures of Col-0, *perk13.1,* and *perk13.1* complemented lines in the absence and presence of *P. annua*, surrounded by grey and red squares, respectively. (C) Barplots of the Least Square means of the HD ratio in the absence and presence of *P. annua* expressed as a percentage of Col-0 in the absence of *P. annua*. FDR corrected *p*-values between the absence and presence of *P. annua* for each genotype: *0.05 > *P* > 0.01, **0.01 > *P* > 0.001, *** *P* < 0.001, absence of symbols: non-significant.

The competitive response to *P. annua* depends on *PERK13* sequence variability in natural accessions

To gain insights into a potential relationship between *ESC1/PERK13* natural sequence diversity and its role in response to *P. annua* presence, we selected eight TOU-A accessions used in the initial GWAS. We chose them according to their contrasting response to *P. annua* as well as their allele for the most associated SNP at the position 26,555,224 on chromosome 1, with four accessions chosen for each allele (**Figure 2A**). The sequencing of the eight TOU-A accessions and the re-sequencing of Col-0 for a ~5.6kb region encompassing the promoter and the coding regions of *PERK13*, revealed 93 indels and 88 SNPs (**Figure 2A**). The majority of

polymorphisms were located in the kinase domain (49%) and the promoter region (38%), while none were detected in the transmembrane or extracellular domains of *PERK13* (**Figure 2A**). Strikingly, all the 17 polymorphisms located in the exons correspond to synonymous mutations. We identified three distinct haplotypes among the eight TOU-A accessions (**Figure 2A**). While Haplotype 1 and Haplotype 2 are closely related to Col-0, Haplotype 3 differs significantly from them, particularly in the kinase domain region, where the 88 identified polymorphisms are in complete linkage disequilibrium.

To test whether these highly divergent haplotypes mediate different competitive responses to the presence of *P. annua*, we complemented the mutant *perk13.1* with *PERK13*-Haplotype 2 and *PERK13*-Haplotype 3 (**Figures 2B** and **2C**). We observed that the three lines complemented with *PERK13*-Haplotype2 showed a significant increase in the HD ratio in response to the presence of *P. annua*, similar to Col-0 or the lines complemented with the Col-0 haplotype of *PERK13* (**Figure 2B**). In contrast, the three lines complemented with *PERK13*-Haplotype3 exhibited similar HD ratios in both the absence and presence of *P. annua* (**Figures 2B** and **2C**, **Table S3**. Together, these results demonstrate the existence of a functional haplotype (Haplotype 2) and a defective haplotype (Haplotype 3), thereby confirming the role of *PERK13/ESC-1* in the natural variation of the HD ratio in response to the presence of *Poa annua*.

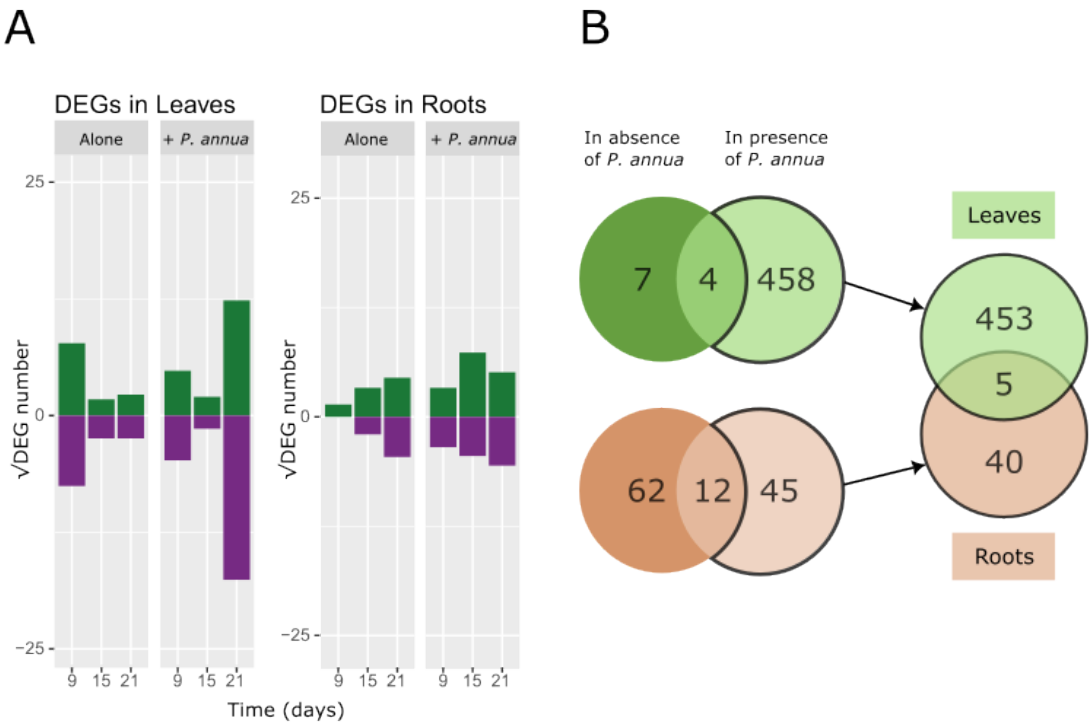
The *ESC1/PERK13*-dependent response to *P. annua* competition relies on stress-related genes

To investigate the molecular pathways involved in the escape strategy mediated by *ESC1/PERK13*, we conducted an RNAseq experiment on 9, 15, and 21 day-old *A. thaliana* roots or leaves of *perk13.1*, *PERK13*Ox, and Col-0 grown in the presence or absence of *P. annua*. We compared the transcriptional reprogramming in *perk13.1* or *PERK13*Ox to the transcriptional reprogramming in Col-0 at each developmental stage and in each compartment

to identify differentially expressed genes (DEGs). In the presence of *P. annua*, we observed distinct transcriptomic changes between leaves and roots in the *perk13.1* mutant. For instance, in 21-day-old plants grown in the presence of *P. annua*, 462 DEGs were identified in the leaf compartment, while only 57 DEGs were identified in the root compartment. Notably, only 5 genes were identified as commonly deregulated in the two compartments in response to *P. annua* (**Figure 3A** and **3B**). Moreover, while the number of DEGs across development stages was relatively stable in the root compartment, regardless of the absence or presence of *P. annua*, a sharp increase in the number of DEGs was observed in the leaf compartment during the development of *A. thaliana*, especially in presence of *P. annua* (**Figure 3A**). Compared to the *perk13.1* mutant, we observed a higher number of DEGs for the overexpressing line in the two plant compartments whatever the absence or presence of *P. annua* (**Figures S1** and **S2**). Consequently, we focused our further analysis on the DEGs of the *perk13.1* mutant in 21-day-old plants, since most of the genes were deregulated at this developmental stage. In addition, we only kept the genes differentially expressed specifically in the presence of *P. annua*. This led to the final sets of 458 and 45 DEGs in the leaf and root compartments, respectively (**Figure 3B)**.

To understand more precisely the molecular pathways driven by *ESC1*/*PERK13* in response to *P. annua,* a GO term enrichment analysis was performed on these two lists of genes revealing a global reprogramming primarily centered on stress responses. In the leaf compartment, while DEGs were related to diverse biological processes responses such as circadian rhythm and cell communication, a clear enrichment was observed in many categories related to the response to either biotic or abiotic stimuli (**Figure 3C**). In the root compartment, a few functional categories also related to stress were enriched. Interestingly, in both compartments, several DEGs were associated with plant immune responses, including (i) perception related genes such as LRR protein kinases, (ii) signaling related genes including

kinases, calcium and ROS related genes, (iii) transcription factors, and (iv) genes related to metabolism and hormone regulation (**Table S5**).



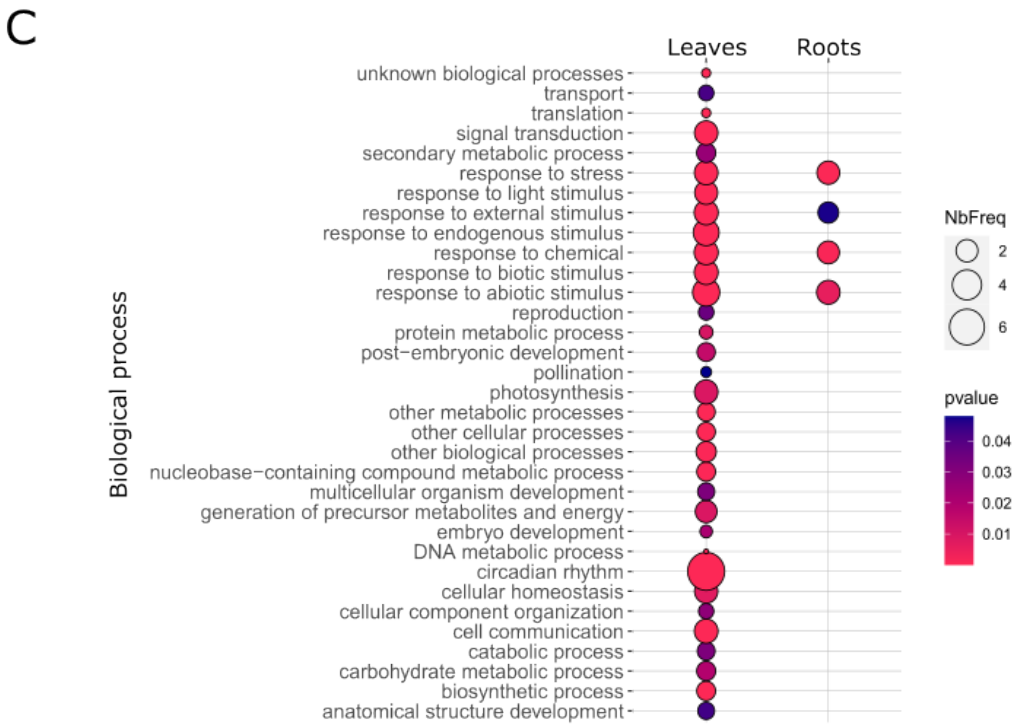


Figure 3. Transcriptional reprogramming in *perk13.1* in response to *P. annua*. (A) Upregulated genes (green) and downregulated genes (purple) found in the *perk13.1* mutant in comparison to Col-0 for each treatment, tissue, and timepoint. (B) Venn diagrams representing the DEGs found in 21-day-old plants in the absence (dark color) and the presence of *P. annua* (light color), for the leaf compartment (green) and the root compartment (orange). The parts of the diagram outlined in bold represent the DEGs

specific to *P. annua* response and are used for DEGs comparison between the leaf and root compartments in (C). (C) Go terms enriched in leaves and roots of 21-day-old plants of the *perk13.1* mutant in the presence of *P. annua*. *P*-values < 0.05 were selected and the Normed Frequency (NbFreq) was calculated as follows: (Number_in_Class input/Number_Classified input)/(Number_in_Class reference/Number_Classified reference).

ESC1/PERK13 is part of two decentralized protein networks

Our transcriptomic analysis allowed the identification of *ESC1/PERK13*-dependent genes that were deregulated in response to the presence of *P. annua* and that might be important for setting up an escape strategy. To gain a broader understanding of the complex response of *A. thaliana* to the presence of *P. annua* and identify key regulatory components, we reconstructed protein–protein interaction networks using the sets of DEGs identified in our transcriptomic analysis conducted on the leaf and root compartments. In addition, we performed a Yeast-Two-Hybrid (Y2H) screen using a library generated from *A. thaliana* plants grown in the presence of *P. annua*, enabling the identification of candidate proteins interacting with the ESC1/PERK13 kinase domain. Among the 15 candidate proteins identified (**Table S6**), four of them were identified at high frequency and correspond to two calcium-related proteins [Calmodulin-binding Receptor-like Cytoplasmic Kinase 2 (CRCK2) and CDPK-related protein Kinase 4 (CRK4)], a Vascular plant One Zinc finger protein (VOZ1), and Lysophospholipase 2 (LysoPL2). Reconstruction of our interaction networks was performed using these candidate proteins, along with the DEGs, and by looking for known interactors of the proteins encoded by the DEGs and of the ESC1/PERK13 putative interactors identified in this study.

The leaf protein-protein interaction (PPI) network consists of 3032 interactions between 1502 proteins (nodes) (**Figure 4**, **Table S7**). Clustering coefficients are close to 0, indicating that nodes are quite scattered across the network and not grouped into clusters. The average betweenness is relatively low, suggesting a limited number of central nodes. Additionally, 28% of the network members are connected with at least 3 neighbours, 7% are connected to more

than 10 proteins, and 92% of the proteins (N = 1385) are linked to PERK13 through PPI. These data reveal a complex and intricated PPI network, with a consistent part of this network corresponding to genes presenting an ESC1/PERK13-dependent expression (56% of the DEGs). (**Figure 4** and **Table 1**). The organization of the network is composed of modules not related to specific functions, which contrasts with previous observations in studies conducted on other types of biotic interactions, such as plant-pathogen interactions (Delplace et al. 2022; **Figure 4** and **Table S7**). Using the five main classification methods in cytoHubba, we identified hub and bottleneck proteins, with the top ten from each method listed in **Table S8**. Four main hubs were identified in the network of the leaf compartment: MYC2 (AT1G32640), MYB73 (AT4G37260), JAZ1 (AT1G19180), and GI (AT1G22770) (**Figure 4** and **Table S8**). Interestingly, these four genes are mainly known for their role in response to biotic or abiotic stresses (Fornara et al. 2015; Gautam et al. 2021; Wang et al. 2021; Zhao et al. 2024).

The root network exhibits similar characteristics to the leaf network, despite its smaller size, comprising 599 nodes and 696 interactions (**Figure S3**, **Table S10** and **S11**). Interestingly, ESC1/PERK13 appears to be more central and the second main hub in the root compartment (**Figure S3 and S4**).

Table 1. General network topology measurements for Leaves and Roots ESC1-dependent networks in response to the presence of *P. annua*. All data were calculated using Network Analyzer of Cytoscape.

Network	Average degree	Average betweenness	Clustering coefficient	Diameter	Average path length	Number of nodes	Number of edges
Leaves	4.03	0.015	0.033	13	5.24	1502	3032
Roots	2.32	0.012	0.006	10	3.81	599	696

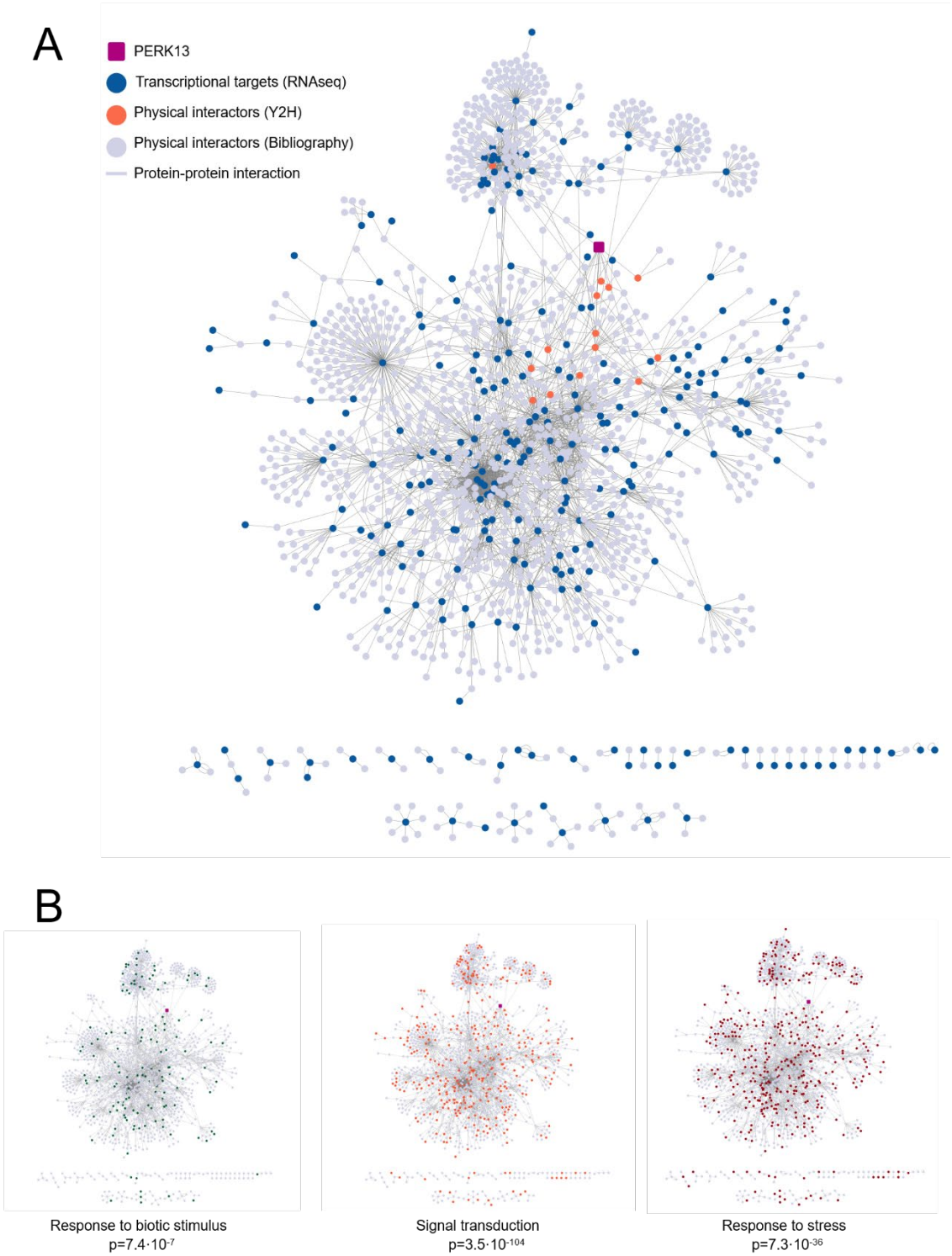


Figure 4. Protein-protein interaction network in leaves is highly interconnected and composed of proteins related to immunity pathways. (A) PERK13 protein-protein interaction networks plotted with Cytoscape showing components used to generate it: PERK13 (magenta), PERK13 physical Y2H partners (orange), proteins identified in the RNAseq analysis (blue) and experimental interactors of DEGs and Y2H proteins (grey). (B) Enriched main functional classes in this network showing in color proteins assigned to said category and *p*-value associated.

Discussion

The molecular mechanisms underlying plant competitive response remain poorly understood. Yet, understanding these mechanisms could help to predict the dynamics of natural plant communities in ecological time (Pierik et al. 2013; Frachon et al. 2017) and to accelerate breeding programs for the development of crop varieties with enhanced competitive ability against weeds (Worthington and Reberg-Horton 2013; Becker et al. 2023). In our study, we focused our analysis on the escape strategy of *A. thaliana* in response to the neighboring plant *P. annua*, identified as one of the main species occurring in *A. thaliana* natural populations (Frachon et al. 2017, 2019).

ESC1/PERK13,* a gene underlying the natural variation of interspecific competitive response in *A. thaliana

Previously fine-mapped in a GWAS, a QTL associated with natural variation in the escape strategy, served as the starting point for identifying genes related to plant response to the presence of a competitor. By combining genetic and molecular approaches, we functionally validated the gene underlying this QTL, which encodes a proline-rich extensin-like receptor kinase (PERK), *ESC1/PERK13.* To our knowledge, this is the first gene underlying the natural variation of the response to interspecific competition that has been cloned. In the broader context of interspecific plant-plant interactions, only six genes, all conferring resistance to a parasitic plant, have been cloned and functionally validated to date (Li and Timko 2009; Cardoso et al. 2014; Hegenauer et al. 2016; Gobena et al. 2017; Duriez et al. 2019).

Interestingly, *ESC1/PERK13* shares similar eco-genomic patterns with genes involved in plant immunity. For instance, as previously observed for disease resistance genes such as the *NLR* gene *RPS5* and the atypical kinase *RKS1* (Huard-Chauveau et al. 2013; Karasov et al. 2014;

Frachon et al. 2017), *ESC1/PERK13* exhibits two highly divergent haplotypes (Haplotype 1 + Haplotype 2 *vs* Haplotype 3) within the French local mapping population TOU-A. The maintenance of this long-lived polymorphism may result from either a fitness cost associated with one of the two haplotypes, conditioned by the presence of competitors, or each haplotype is beneficial in the face of different competitors. In our study, one of the two haplotypes is involved in the response to the presence of *P. annua* and *T. aestivum*. Whether Haplotype 3 is involved in the response to different competitors remains to be tested.

ESC1/PERK13 may mediate plant competitive response by perceiving environmental modifications

ESC1/PERK13 is a plasma membrane protein anchored in the cell wall, which is a negative regulator of root hair growth (Won et al. 2009; Hwang et al. 2016). In the context of plant-plant interactions, this localization suggests that ESC1/PERK13 may trigger an adaptive response by perceiving neighboring plants (either directly or indirectly). Interestingly, PERK13 was recently shown to modulate root growth under phosphate deficiency (Xue et al. 2021). We may therefore conceive that nutrient depletion triggered by the proximity of the roots of *P. annua* might be at the origin of the ESC1/PERK13-mediated competitive response through an initial perception at the root level. This hypothesis would be in line with the common assumption that plants passively perceive their neighbors by detecting the changes in their immediate environment (Novoplansky 2009). Previous transcriptomic studies indeed revealed the implication of light perception and nutrient-dependent signaling pathways as key components of the plant competition response (Geisler et al. 2012; Schmid et al. 2013; Horvath et al. 2015). However, although light perception and nutrient-related pathways were present in our transcriptomics analysis, biotic stress responses and their associated signaling pathways were identified as the predominant biological processes mediating the response to *P. annua*.

The implication of genes related to pathogen response is consistent with the identification of several RLKs in association genetics studies performed across the genome of *A. thaliana* in the context of interspecific interactions (Frachon et al. 2019; Libourel et al. 2021). These RLKs include Flagellin-Sensing 2 (FLS2), BRI1 Associated Receptor Kinase (BAK1), and BAK1-Interacting Receptor-Like Kinase 1 (BIR1), which belong to distinct subfamilies and participate in different aspects of plant immunity. Their involvement suggests that multiple layers of receptor-mediated signaling, including perception, signal integration, and regulation, could be recruited during plant-plant interactions, supporting the view of a complex and coordinated active plant response. ESC1/PERK13 belongs to the PERK gene family comprising 15 members in *A. thaliana,* in which it is closely related to the pollen-specific *PERK11* and *PERK12* genes (Nakhamchik et al. 2004; Invernizzi et al. 2022). While PERKs have traditionally been linked to developmental processes (Borassi et al. 2016), emerging evidence indicates a broader role as sensors of abiotic and biotic cues (Invernizzi et al. 2022). For instance, while Z15 has been implicated in rice tolerance to low temperatures (Feng et al. 2019), the PERK GmHAK2 was identified as a component of the response to herbivores in soybean (Uemura et al. 2020), and four *BnPERKs* were downregulated in *Brassica napus* during *Plasmodiophora brassicae* infection (Zhang et al. 2025). However, their mode of action, notably the identification of their ligands, remains elusive, although PERKs have been hypothesized to be associated with cell wall compounds because of their extracellular proline-rich domain (Nakhamchik et al. 2004; Humphrey et al. 2015). Identification of the ligand, directly or indirectly produced by *P. annua*, that induces the escape strategy in *A. thaliana*, would therefore help for a better understanding of the *PERK13* functions. RLKs interact with one or several co-receptors to fulfill their functions. In this context, the homodimerization of GmHAK2, involved in soybean defense responses to an herbivore, was demonstrated (Bai et

al. 2009). Still, a screen for interactors using the PERK extracellular domain has not been reported yet.

ESC1/PERK13 mediates competitive response by distinct root and leaf transcriptomic responses

Based on our transcriptomic analysis, the presence of *P. annua* appears to trigger two distinct reprogramming responses in *A. thaliana* between roots and leaves. This is well illustrated by the few number of common DEGs as well as the few biological functions enriched in both compartments. However, biotic and abiotic stresses responses are triggered in both leaves and roots. Interestingly, the ESC1/PERK13-mediated transcriptional changes are more pronounced in leaves than in roots, aligning with the observed aboveground phenotype (i.e., the HD ratio), despite *ESC1/PERK13* expression having been previously reported primarily in the root compartment (Hwang et al. 2016). The smaller number of genes differentially expressed in roots, mostly linked to perception and transduction, could suggest a key role of this compartment in the perception of *P. annua* and the transfer of a putative signal in the shoot compartment. Accordingly, the overexpression of *ESC1/PERK13* in roots (PERK13Ox) induces an important transcriptomic response in roots, both in the absence and presence of *P. annua*, but also in leaves with 1578 down-regulated and 1165 up-regulated genes specifically in the presence of *P. annua* (Figure S2). This signal, possibly carried by peptides or microRNAs, could trigger genetic reprogramming in the leaves, thereby leading to a reallocation of resources to stem growth and flowering (Tsikou et al. 2018; Lebedeva et al. 2020; Dong et al. 2022; Zhang et al.). In *Populus euphratica*, a similar expression pattern has been observed in response to drought, where root and leaf responses were distinct, with major transcriptional reprogramming occurring in the leaves to regulate photosynthesis (Jiao et al. 2021). Interestingly, the limited overlap between responses observed in roots and leaves was also observed during intraspecific competition in *A. thaliana* (Masclaux et al. 2012). In this

study, photosynthesis-related genes were regulated in the leaves, while nutrient-related genes were regulated in the roots. However, it should be underlined here that biotic stress and defense pathways were activated in both compartments, although the specific genes involved differed. Collectively, these observations suggest a perception of the presence of a neighboring species at the root level, followed by an above-ground transcriptional reprogramming, both processes implicating some functions related to response to biotic stresses.

ESC1/PERK13 as an entry node to induce a plant competitive response

Years of global studies have produced extensive data, requiring integration and analysis to better understand the mechanisms involved (Becker et al. 2023). Thus, systems biology approaches have been employed to gain deeper insight into the regulation of processes involved in the response to environmental cues, such as pathogen attacks, ultimately leading to the identification of novel immune pathways (Tsuda and Katagiri 2010; Brauer et al. 2018; Delplace et al. 2022). Identification of DEGs and ESC1/PERK13 interactors by Y2H screening allowed us to generate two protein-protein interaction networks highlighting the ESC1/PERK13-dependent responses to *P. annua* in the leaf and root compartments. Consistent with our transcriptomics analysis, the two networks displayed a substantial difference in the number of proteins. Nonetheless, in both networks, we identified overrepresented functions, such as signal transduction, shoot system development, and cell communication. These functions are often organized into highly connected modules in other biotic stress-generated networks (Delplace et al. 2020). For exemple, in the case of PTI, four major hormonal sectors were identified which, through compensatory mechanisms under pathogenic perturbations, resulted in network robustness (Kim et al. 2014; Hillmer et al. 2017). In our study, the nodes of the networks are more dispersed than clustered. The presence of a scale-free topology, combined with functional redundancy, likely contributes to network stability under environmental perturbations (Rodrigues 2025).

Still, both networks possess major hubs that may be important for network function (Vandereyken et al. 2018). For instance, TCP14 is highly connected to many transcription factors and has been identified in pathogen susceptibility networks (Mukhtar et al. 2011; Weßling et al. 2014). ESC1/PERK13 appears as a bottleneck protein in our study, suggesting it might connect different nodes and modules, possibly influencing the flow of the network. As a receptor potentially acting as a bridge between external stimuli and intracellular responses, ESC1 might indeed have a crucial position in the network. Such a pattern has previously been shown for other receptor proteins (Ahmed et al. 2018). However, ESC1/PERK13 interacts only with a few proteins in the network proteins including fifteen Y2H putative interactors identified in our study, and one detected in another analysis, KIPK1 (Humphrey et al. 2015), in comparison, for instance, with the well-known FLS2 or BAK1 receptors that have many interactors (Ahmed et al. 2018). This could be explained by the lack of data available on plant-plant interactions in comparison with other types of biotic interactions, thereby limiting the network complexity. It could also be possible that the main interactors of ESC1 belong to upstream events of genetic reprogramming. This reinforces the importance of combining methods for identifying signaling pathways and the importance of finding interacting partners of proteins.

Conclusion

Our work provides the first example of the identification and validation of a gene involved in the natural variation of plant response to competition by a weed species. While plant-plant interactions are thought to be mainly driven by competition for light, nutrients, and water (Pierik et al. 2013), we demonstrated in this study that *A. thaliana* could directly or indirectly sense and respond to specific neighborhoods through receptor-ligand proteins, as previously shown in plant-parasitic plant interactions (Li and Timko 2009; Hegenauer et al. 2016; Duriez et al.

2019). In addition, we found that many genes associated with plant defense and stress responses were mobilized as part of the escape strategy, reinforcing the concept of an "active" perception and response to a plant competitor.

Materials and Methods

Plant materials

The T-DNA mutant lines of *A. thaliana* used in this study were ordered to the Nottingham *Arabidopsis* Stock Centre (NASC, http://Arabidopsis.info/BasicForm) and are in the Col-0 background (**Table S1**). One T-DNA mutant line is a GABI-Kat line (GK-345C10) whereas the remaining T-DNA mutants were identified in the SALK library (http://signal.salk.edu). The position of the T-DNA insertion was confirmed by polymerase chain reaction (PCR) using LP and RP primers designed using the online T-DNA Primer Design tools (http://signal.salk.edu/tdnaprimers.2.html) and the specific left border primer T-DNA insertion LBb1.3 (**Table S9**). Amplicons were sequenced using specific LP, RP, and LBb1.3 primers and assembled using Phred, Phrap, and Consed software. The results from sequencing were online BLAST using the web interface provided by NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the T-DNA insertional position in the genome. Seeds of the overexpressor line of *PERK13*, named *PERK13*Ox, were kindly provided by Prof. Hyung-Taeg Cho (Seoul National University, South Korea). This line, generated in the Col-0 background, contains the *PERK13* coding sequence under the control of the *EXPANSIN A7* promoter (Cho and Cosgrove 2002) leading to an overexpression in root hairs. To reduce maternal effects, seeds of all these lines were produced under the same greenhouse conditions.

For Y2H experiments, *Arabidopsis* Col-0 wild-type plants were sown on 100 squared petri dishes containing MS medium with the competitor *Poa annua* in a growth chamber at 21°C with a 16h photoperiod.

In this study, we also used the annual bluegrass *Poa annua* (Poaceae) as a neighboring species and six other species, namely the chickweed *Stellaria media* (Caryophyllaceae), the speedwell *Veronica arvensis* (Plantaginaceae), the Kentucky bluegrass *Poa pratensis* (Poaceae), the cat grass *Dactylis glomerata* (Poaceae), the oat *Avena sativa* (Poaceae), the common wheat *Triticum aestivum* (Poaceae) (**Table S10**). Seeds for the first four species were obtained from the Arbiotech Company (http://www.arbiotech.com). Seeds for *A. sativa* and *T. aestivum* were kindly provided by Dr. Etienne-Pascal Journet (AGIR, INRAE, Castanet-Tolosan, France).

PERK13 **sequencing, plasmid constructions, and transgenic plant generation**

The *PERK13* gene and its flanking regions (~5.6kb) from the Col-0 accession and eight accessions of the local TOU-A mapping population (A1-115, A1-79, A6-104, A1-124, A6-61, A6-107, A1-120 and A6-27, Figure 2A), were sequenced after amplification with the RHS10_LR_Fwd and RHS10_LR_Rv primers using the PrimeSTAR® GXL DNA Polymerase (Takara) (**Table S9**). The sequencing was performed by Sanger technology using 23 primers (RHS10_LR_X) to cover the 5.6kb region (**Table S9 & Supplementary File 1**). Sequences were assembled using the Phred, Phrap, and Consed softwares. The results were online BLAST using the web interface provided by NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

To generate the constructs for complementation experiments, amplicons obtained for Col-0, A1-124, and A6-61 with the primers attB1_primer and attB2R_primer (**Table S9**) were cloned into the donor vector pDONR207, using multisite Gateway technology (Life Technologies). Subsequently, the respective constructs were cloned into the pEG301 vector and introduced in *Agrobacterium tumefaciens* (strain GV3101) by electroporation. Three-week-old *perk13.1* loss-of-function plants were transformed by floral dip (Clough and Bent 1998). For each construct, at least three independent homozygous lines were selected for phenotyping and molecular characterization.

Measurement of aboveground traits

Experimental design. Phenotyping experiments were replicated three times for each line used in this study (**Table S11**). For phenotyping of the T-DNA mutant lines and the complemented lines, we used for each replicate a split-plot design arranged as a randomized complete block design (RCBD) with two competition treatments (i.e. absence and presence of *P. annua*) nested within blocks (**Table S11**). We included Col-0 as a control in each experiment (**Table S11**). For testing the specificity of the competitive response mediated by *PERK13* towards other plant species than *P. annua*, we used a split-plot design arranged as a randomized complete block design (RCBD) with eight competition treatments (i.e. absence and presence of seven different species, nested within blocks (**Table S4**).

Growth conditions. The experiments were conducted in the same growth chamber of the Toulouse Plant-Microbe Phenotyping Platform (TPMP, https://eng-phenotoul.hub.inrae.fr/who-we-are/phenotoul/tpmp). Pots (7cm x 7cm x 6cm) were filled with damp standard culture soil (PROVEEN MOTTE 20, Soprimex). In presence of neighboring species, each *A. thaliana* plant was surrounded by three neighboring plants. Seeds for neighboring plants were evenly spaced, 2 cm away from the *A. thaliana* central position. During the experiments, plants were grown at 20 °C under artificial light to provide a 16-hr photoperiod and were bottom watered without supplemental nutrients. *A. thaliana* focal seedlings and neighbor seedlings were thinned to one per pot 6 to 12 days after seed sowing. The germination date of *A. thaliana* target seedlings was daily monitored.

Phenotypic traits. Two raw phenotypic traits were measured on each focal plant of *A. thaliana* at the time of their flowering, which was measured as the number of days between germination and flowering date. The first trait corresponds to the height from the soil to the first flower on the main stem (H1F expressed in mm). H1F is related to seed dispersal (Wender et al. 2005) and shade avoidance (Dorn et al. 2000) in *A. thaliana*. The second trait corresponds to the

maximum diameter of the rosette, which was measured at the nearest millimeter (DIAM) (Weinig et al. 2006). This trait is a proxy of the growth of the rosette of the focal plant from germination to flowering. These traits allowed us to estimate the HD ratio as the height from the soil to the first flower on the rosette diameter (i.e. H1F/DIAM).

Statistical analysis. The following mixed model (PROC MIXED procedure, REML method, SAS 9.3, SAS Institute Inc) was used to explore the phenotypic differences among the different lines:

$$Y_{iikl} = \mu_{\text{trait}} + \text{Replicate}_j + \text{Block}_i\,(\text{Replicate}_j) + \text{Treatment}_k + \text{Line}_l + \text{Block}_i \times \text{Treatment}_k + \text{Treatment}_k \times \text{Line}_l + \text{Treatment}_k \times \text{Replicate}_j + \text{Replicate}_j \times \text{Treatment}_k \times \text{Line}_l + \varepsilon_{iikl} \quad (1)$$

where '*Y*' is the HD ratio scored on focal *A. thaliana* plants, 'μ' is the overall phenotypic mean; 'Replicate' accounts for differences among the temporal replicates; 'Block' accounts for environmental variation among experimental blocks within each replicate; 'Treatment' corresponds to the effect of the presence of a neighboring species (absence of competitor *vs* presence of *P. annua, V. arvensis, S. media, P. pratensis, D. glomerata, A. sativa,* and *T. aestivum*); 'Line' measures the effect of the different genetic lines; the interaction term 'Treatment' x Line' accounts for variation among genetic lines for their reaction norms across the treatments. All factors were treated as fixed effects. For the calculation of *F*-values, terms were tested over their appropriate denominators. Given the split-plot design used in this study, the variance associated with 'Block x Treatment' was used as the error term for testing the 'Block' and 'Treatment' effects. Least Square means (LSmeans) of the HD ratio were obtained for each 'Treatment x Line' combination following the model (1).

Y2H library construction and screening

Saccharomyces cerevisiae strains Y2H Gold and Y187 were grown at 30°C on YPDA medium and were used for bait and prey library cloning respectively. RNA extraction was performed from *A. thaliana* plants (Col-0) harvested at 4, 10 and 14 days (at least 3 plants/sample) after

sowing and in presence of the plant competitor *P. annua* (3 independent experiments). RNA quality and integrity were checked using RNA 6000 Nano chip (Agilent, A_{260}/A_{280} ~2, A_{260}/A_{230} = 2.0-2.2 and RIN>6). The library construction was performed from RNA samples from each timepoint, which were subsequently pooled in equimolar ratio and using the Make Your Own "Mate & Plate™" Library System (Takarabio, 2021). mRNA was purified using Invitrogen Dynabeads™ Oligo$(dt)_{25}$ following the protocol supplied by the manufacturer. The cDNA mix was co-transformed with pGADT7-Rec plasmid in competent Y187 cells using the Yeastmaker Yeast Transformation System 2 (Takarabio, 2010).

For library screening, the PERK13 Kinase domain (KD) was used after PCR amplification (**Table S9**) for cloning into pGBG-GWY expression vector and transformation of *S. cerevisiae* Y2HGold strain (AUR1-C, ADE2, HIS3, and MEL1). Yeast transformants were selected on SD media lacking tryptophan, leucine and histidine (-TLH) and lacking also Adenine (-TLHA). Candidate interactors were identified by sequencing using specific pGAT-T7-Rec primers (**Table S9**). For one-by-one Y2H interactions, plasmids of the candidates were co-transformed with PERK13-KD in Y2H Gold yeasts on SD-LT media and then transferred on SD-TLH media.

Gene expression analysis by RT-qPCR

Total RNA was extracted from 14-day-old plant roots grown under *in vitro* conditions, from eight to nine biological replicates per genotype and three independent experiments, with the NucleoSpin® RNA kit (Macherey Nagel). 500ng of total RNA was used for cDNA synthesis with the reverse transcriptase Transcriptor according to the manufacturer's protocol (Roche E1372). Quantitative RT-PCR reactions were performed in 10µL using SYBR ® Green II master mix (Brilliant II SYBR Green QPCR Master Mix, Sigma-Aldrich). Gene expression was normalized using the housekeeping genes *ACT2* (AT3G18780) and *MON1* (AT2G28390) (Czechowski et al. 2005).

Transcriptomic analysis

Sample preparation and total RNA extraction

A. thaliana leaves and roots grown in the presence or absence of *Poa annua*, were sampled at 9, 15 and 21 days after sowing and flash frozen in liquid nitrogen. Three replicates of 3 pooled plants were sampled for each timepoint except for the 1st timepoint, where 12 plants were pooled. Three independent experiments were conducted, and total RNA extraction was performed using Nucleospin® RNA plus kit (Macherey-Nagel) and quality was checked using RNA 6000 Nano Kit (Agilent).

RNA sequencing and analysis

RNA sequencing was outsourced to Novogene (Cambridge, United Kingdom) to produce Illumina 150 bp paired-end Illumina using NovaSeq6000 sequencing platform. Raw paired-end reads were processed using the nf-core/rnaseq pipeline version 3.0 (doi:10.5281/zenodo.4323183). Pseudo-mapping strategy with salmon software (version 1.4.0) and reference genome annotation of *Arabidopsis thaliana* version Araport11 were used. Counts were retrieved at the gene level and normalized using the TMM method from EdgeR package (version 3.38.4). Differential gene expression analysis was performed using R version 4.2.0 (2022-04-22) and the EdgeR_3.40.2 (Robinson et al. 2010). DEG lists were generated by comparing expression in deregulated lines and Col-0 and this for each treatment and time point. From these lists, genes with an FDR < 0,1 were selected and joint to create a new DEGs list at 21 das (**Table S5**). The packages ggplot2 v.3.4.3, ggpubr v.0.6.0 and vegan 2.6.4 were used to generate the graphs (Wickham, 2016; Oksanen *et al.*, 2022; Kassambara, 2023). Gene Ontology analysis was done using Classification SuperViewer Tool w/ Bootstrap (Provart & Zhu, 2003). All GO terms associated with selected DEGs can be found in the **Table S5**.

Network reconstruction

BioGRID protein interaction dataset version 4.4.227 was used to recover interactors of the 503 deregulated genes found by transcriptomic analysis, and of the 14 candidate interactors of PERK13 identified by Yeast two-Hybrid screens. Cytoscape software v3.10.1 was used to plot protein-protein interactions, and cytoHubba application was used to calculate the network connectivity (Maere et al. 2005; Lin et al. 2008; Chin et al. 2014). Data used to build the networks are found in the **Table S7**.

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Data Accessibility

Raw phenotypic data will be available in the Dryad database: doi:10.5061/dryad.XXX. RNA-seq reads have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject SRP569129.

Author contributions

F.R., M.H. and D.R. supervised the project. F.R., M.H., D.R., and C.L. designed the experiments. C.L. and F.R. conducted the phenotyping experiments. C.L. analyzed the phenotypic traits. M.I. conducted and analyzed the Y2H screenings. M.H. prepared the samples

for RNAseq. M.I. performed the RNAseq analysis and the network recontruction. C.L., M.I., M.H., F.R., and D.R. wrote the manuscript.

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Supplemental Information

An *Arabidopsis* receptor-like kinase mediates competitive plant-plant interactions

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4 Supplementary **Figure**s

11 Supplementary **Table**s

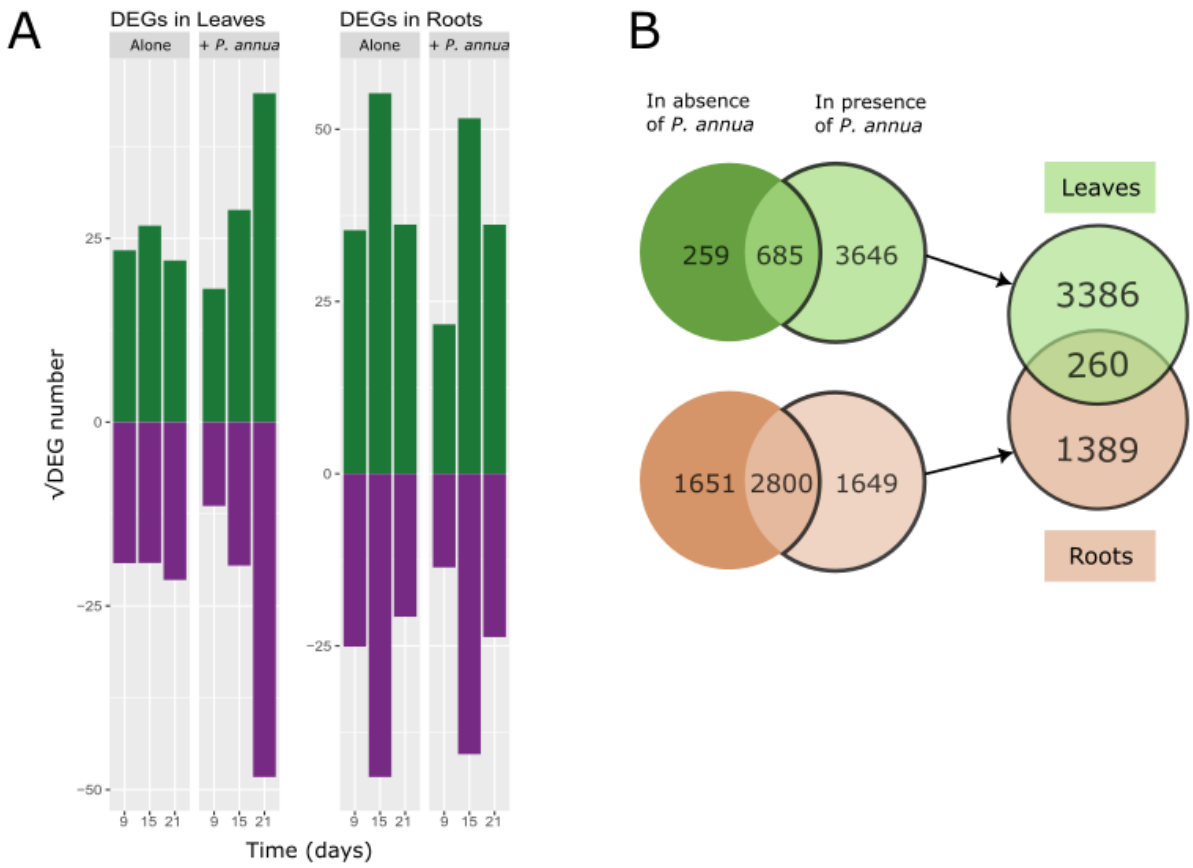

Figure S1. Transcriptionnal reprogramming in the overexpressing line across time and in response to *P. annua*. (A) Upregulated genes (green) and downregulated genes (purple) found in the *PERK13*Ox in comparison to Col-0 for each treatment, tissue and timepoint. (B) Venn diagrams representing the DEGs found in21-day-old plants in the absence (dark color) and in the presence of *P. annua* (light color), for leaves (green) and roots (orange). The parts of diagram outlined in bold represent the DEGs specific to *P. annua* response and are used for DEGs comparison between leaves and roots.

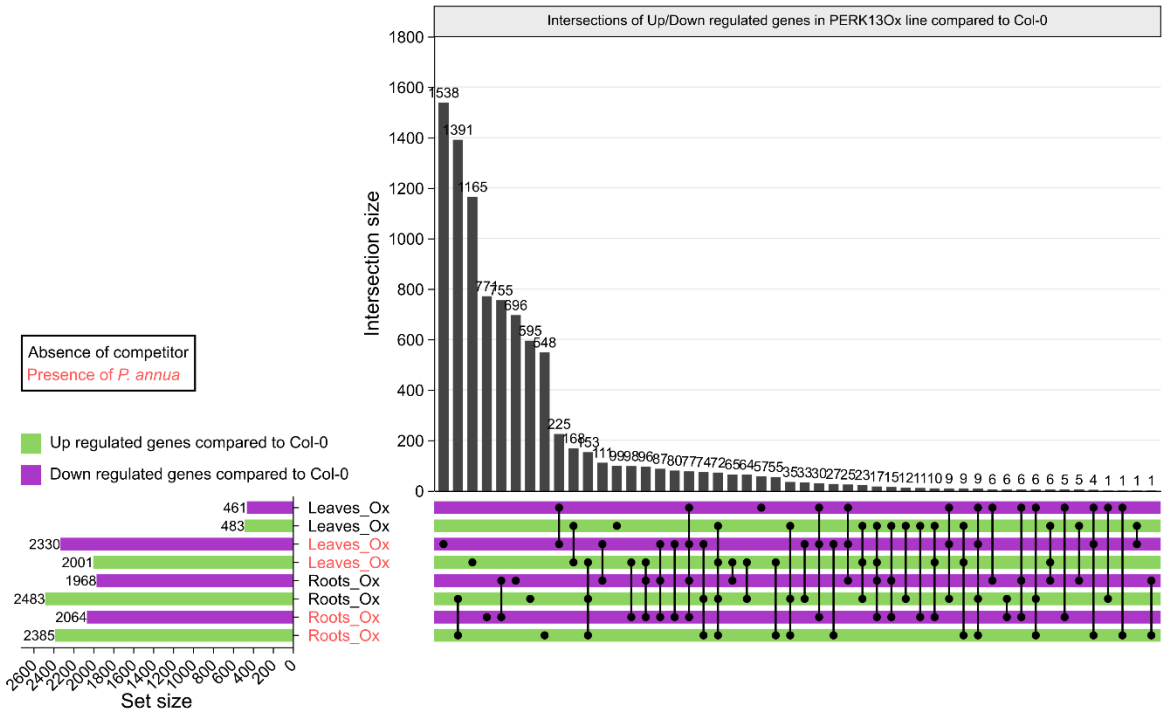

Figure S2. *PERK13Ox* DEGs are affected by the presence of *P. annua* only in leaves. Upset plot of up (green) and down (purple) regulated DEGs of *PERK13*Ox compared to Col-0 identified at 21das in absence (black) or presence (red) of *P. annua*.

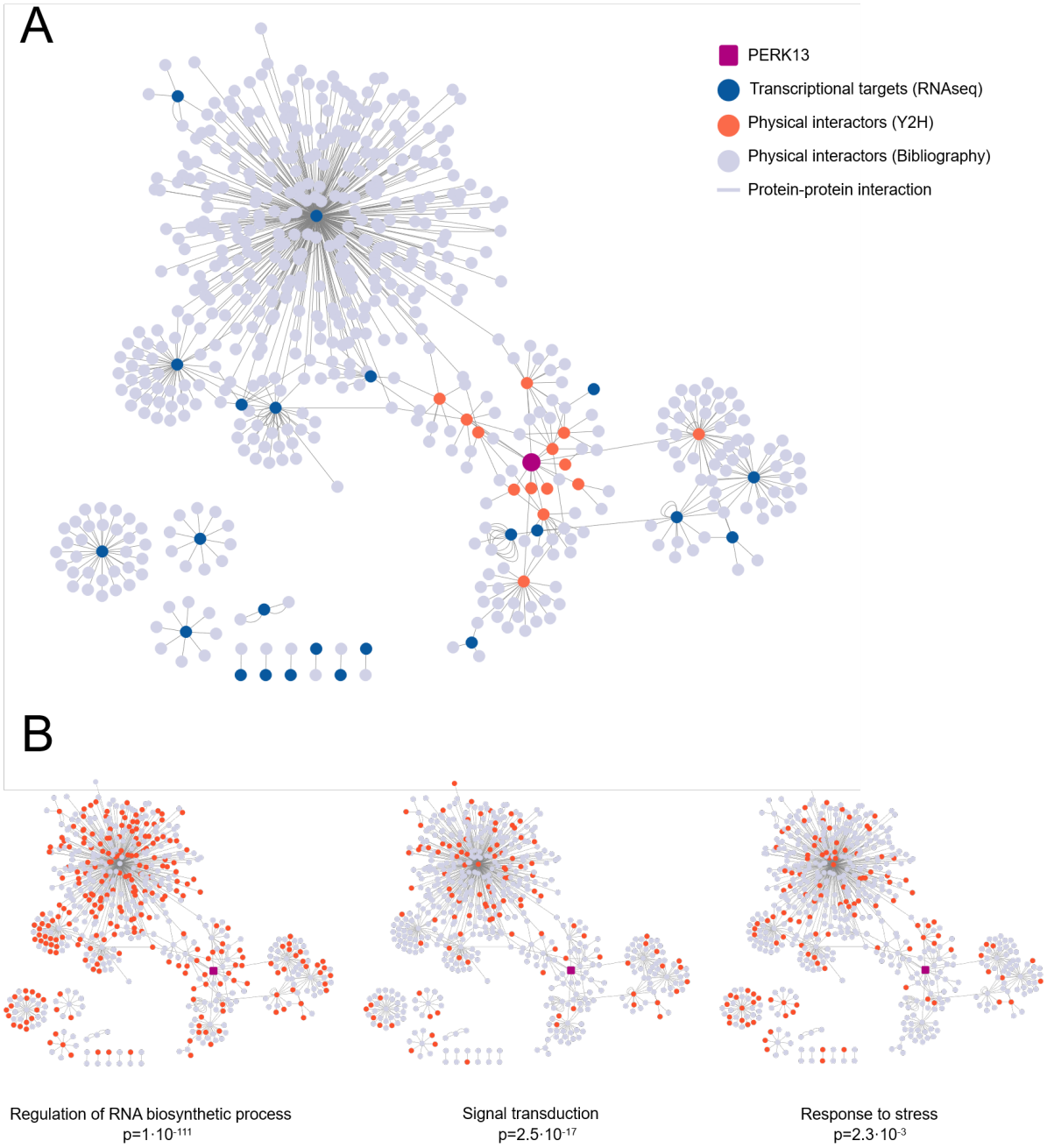

Figure S3. Protein-protein interaction networks in roots is smaller but similar to shoot in terms of classes. (A) PERK13 protein-protein interaction networks plotted with Cytoscape showing components used to generate it: PERK13 (magenta), PERK13 physical Y2H partners (orange), proteins identified in the RNAseq analysis (blue) and experimental interactors of DEGs and Y2H proteins (grey). (B) Enriched main functional classes in this network showing in color proteins assigned to said categories.

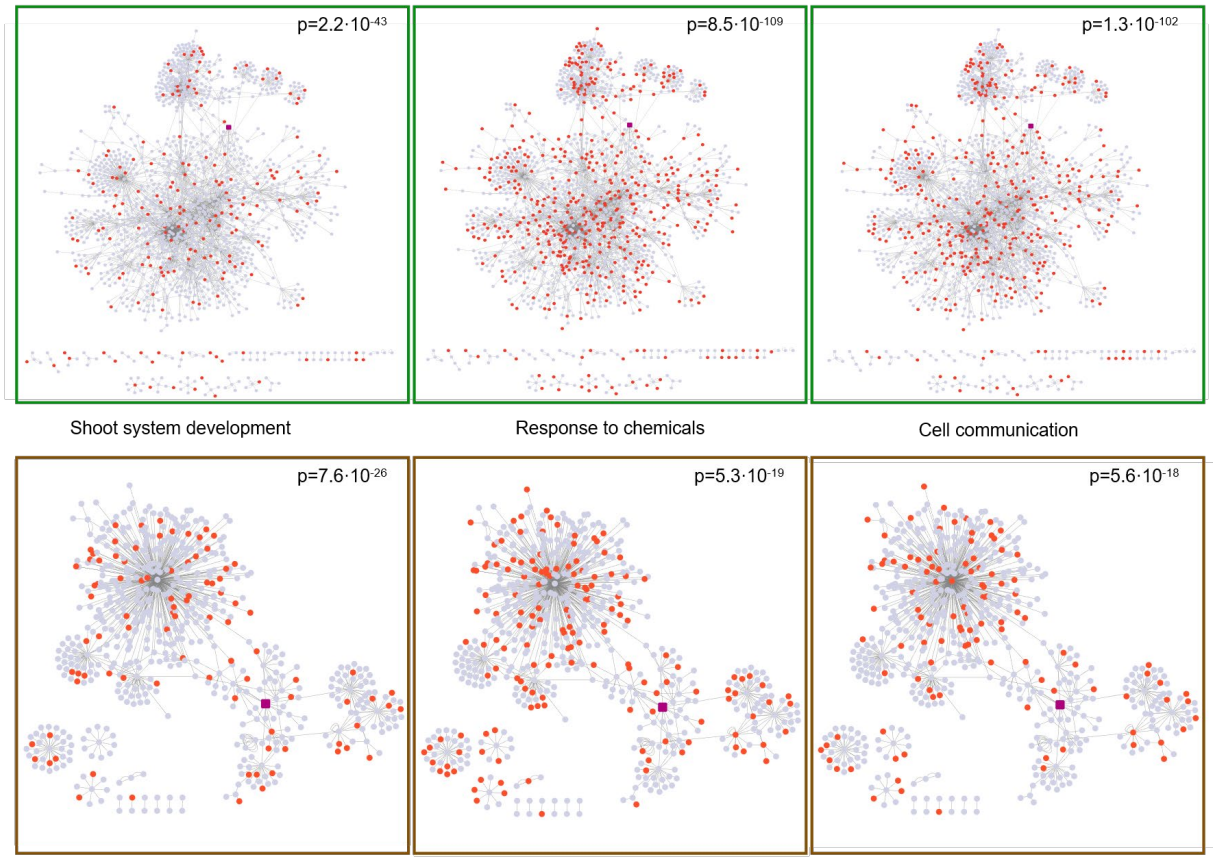

Figure S4. Biological functions are scattered along the two protein-protein interaction networks. GO terms enriched in leaves (green) or roots (brown) networks and associated p-values. Nodes associated with the categories are highlighted in orange.