The calcium sensor CBL7 is required for Serendipita indica -induced growth stimulation in Arabidopsis thaliana, controlling defense against the endophyte and K + homeostasis in the symbiosis

Marta-Marina Pérez-Alonso[1], Carmen Guerrero-Galán[1], Adrián González Ortega-Villaizán[1], Paloma Ortiz-García[1], Sandra Scholz[2], Hitoshi Sakakibara[3], Takatoshi Kiba[3], Jutta Ludwig-Müller[4], Anne Krapp[5], Ralf Oelmüller[2], Jesús Vicente-Carbajosa[1], Stephan Pollmann[1], and Patricio Ramos[6]

[1]Centro de Biotecnologia y Genomica de Plantas
[2]Friedrich Schiller Universitat Jena Institut fur Allgemeine Botanik und Pflanzenphysiologie
[3]Rikagaku Kenkyujo Kankyo Shigen Kagaku Kenkyu Center
[4]Technische Universitat Dresden Institut fur Botanik
[5]Institut Jean-Pierre Bourgin
[6]Universidad de Talca

May 4, 2022

Abstract

Calcium (Ca $^{2+}$) is an important second messenger in plants. The activation of Ca $^{2+}$ signaling cascades is critical in the activation of adaptive processes in response to perceived environmental stimuli, including biotic stresses. The colonization of roots by the plant growth promoting endophyte *Serendipita indica* involves the increase of cytosolic Ca $^{2+}$ levels in *Arabidopsis thaliana*. In this study, we investigated transcriptional changes in Arabidopsis roots during symbiosis with *S. indica*. RNA-seq profiling disclosed the significant induction of *CALCINEURIN B-LIKE 7* (*CBL7*) during early- and later phases of the interaction. Consistent with the transcriptomics analysis, reverse genetic evidence and yeast two-hybrid studies highlighted the functional relevance of CBL7 and tested the involvement of a CBL7-CBL-INTERACTING PROTEIN KINASE 13 (CIPK13) signaling pathway in the establishment of the mutualistic relationship that promotes plant growth. The loss-of-function of *CBL7* abolished the growth promoting effect of *S. indica* and affected the colonization of the root by the fungus. The subsequent transcriptomics analysis of *cbl7* revealed the involvement of this Ca $^{2+}$ sensor in activating plant defense responses. Furthermore, we report on the contribution of CBL7 to potassium transport in Arabidopsis. Triggered by the differential expression of a small number of K $^{+}$ channels/transporter genes, we analyzed K $^{+}$ contents in wild-type and *cbl7* plants and observed a significant accumulation of K $^{+}$ in root of *cbl7* plants, while shoot tissues demonstrated K $^{+}$ depletion. Taken together, our work associates CBL7 with an important role in the mutual interaction between Arabidopsis and *S. indica* and links the CBL7 Ca $^{2+}$ receptor protein to K $^{+}$ transport.

Running title: CBL7 contributes to the symbiosis between Arabidopsis and *S. indica*

Authors:

Marta-Marina Pérez-Alonso[1,2], Carmen Guerrero-Galán[1], Adrián González Ortega-Villaizán[1], Paloma Ortiz-García[1], Sandra S. Scholz[3], Patricio Ramos[4], Hitoshi Sakakibara[5,6], Takatoshi Kiba[5,6], Jutta Ludwig-

Müller[7], Anne Krapp[8], Ralf Oelmüller[3], Jesús Vicente-Carbajosa[1,9] and Stephan Pollmann[1,9*]

[1]Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM)–Instituto Nacional de Investigación y Tecnología Agraria y Alimentación (INIA), Campus de Montegancedo, 28223 Pozuelo de Alarcón (Madrid), Spain

[2]Umeå Plant Science Center, Umeå University, 90736 Umeå, Sweden

[3]Matthias Schleiden Institute of Genetics, Bioinformatics and Molecular Botany, Department of Plant Physiology, Friedrich-Schiller-University Jena, 07743 Jena, Germany

[4]Centro de Investigación de Estudios Avanzados del Maule, Universidad Católica del Maule, 3460000 Talca, Chile

[5]RIKEN Center for Sustainable Resource Science, 1-7-22, Suehiro, Tsurumi, Yokohama, 230-0045, Japan

[6]Department of Applied Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601, Japan

[7]Institute of Botany, Technische Universität Dresden, 01062 Dresden, Germany

[8]Université Paris-Saclay, INRAE, AgroParisTech, Institut Jean-Pierre Bourgin, 78000 Versailles, France

[9]Departamento de Biotecnología-Biología Vegetal, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid (UPM), 28040 Madrid, Spain

*** Correspondence:** Stephan Pollmann stephan.pollmann@upm.es

Keywords: *Arabidopsis thaliana* , Ca^{2+} signaling, Endosymbiosis, Growth promotion, Plant defense, Plant-microbe interaction, Plant performance, *Serendipita indica*

Abstract

Calcium (Ca^{2+}) is an important second messenger in plants. The activation of Ca^{2+} signaling cascades is critical in the activation of adaptive processes in response to perceived environmental stimuli, including biotic stresses. The colonization of roots by the plant growth promoting endophyte*Serendipita indica* involves the increase of cytosolic Ca^{2+} levels in *Arabidopsis thaliana* . In this study, we investigated transcriptional changes in Arabidopsis roots during symbiosis with *S. indica* . RNA-seq profiling disclosed the significant induction of *CALCINEURIN B-LIKE 7* (*CBL7*) during early- and later phases of the interaction. Consistent with the transcriptomics analysis, reverse genetic evidence and yeast two-hybrid studies highlighted the functional relevance of CBL7 and tested the involvement of a CBL7-CBL-INTERACTING PROTEIN KINASE 13 (CIPK13) signaling pathway in the establishment of the mutualistic relationship that promotes plant growth. The loss-of-function of *CBL7*abolished the growth promoting effect of *S. indica* and affected the colonization of the root by the fungus. The subsequent transcriptomics analysis of *cbl7* revealed the involvement of this Ca^{2+} sensor in activating plant defense responses. Furthermore, we report on the contribution of CBL7 to potassium transport in Arabidopsis. Triggered by the differential expression of a small number of K^+ channels/transporter genes, we analyzed K^+ contents in wild-type and *cbl7*plants and observed a significant accumulation of K^+in root of *cbl7* plants, while shoot tissues demonstrated K^+ depletion. Taken together, our work associates CBL7 with an important role in the mutual interaction between Arabidopsis and*S. indica* and links the CBL7 Ca^{2+} receptor protein to K^+ transport.

Significance statement: The induction of *CBL7* is critical for the establishment of the mutual interaction between*Arabidopsis thaliana* and its root colonizing endophyte*Serendipita indica* . Moreover, CBL7 plays a vital role in K^+ distribution during the plant-fungus interaction.

Introduction

Plants have evolved in spatial proximity to a multitude of different microorganisms in their surroundings. Their constant interaction with commensal, symbiotic, and pathogenic microorganisms shaped highly specialized ecosystems in which plants find their niches and flourish. Since the initial description of the concept of mutual coexistence between dissimilar organisms (de Bary, 1879; Hertig et al., 1937), our knowledge regarding the symbiotic associations of plants with microorganisms has substantially advanced. Numerous studies unambiguously demonstrate that plant-microbe interactions are important to the structure, function, and health of plant communities, and that symbiotic fungi contribute to the adaptations of plants to environmental stresses (Rodriguez et al., 2004).

Serendipita indica (formerly termed *Piriformospora indica*) is an axenically cultivable root colonizing plant endophyte of the order Sebacinales with an exceptionally broad host range (Verma et al., 1998; Weiss et al., 2016; Mensah et al., 2020). *S. indica* promotes plant performance and biomass production (Varma et al., 1999; Peškan-Berghöfer et al., 2004; Vadassery et al., 2009), enhances nutrient assimilation (Bakshi et al., 2017; Prasad et al., 2018), and confers increased biotic and abiotic stress tolerance to its host plants (Waller et al., 2005; Sun et al., 2014; Jogawat et al., 2016). The establishment of the symbiosis between *S. indica* and*Arabidopsis thaliana* , and the therewith coupled plant growth promotion, involves the perception of conserved microbial components by the plant, generally termed microbial-associated molecular patterns (MAMPs), through specific pattern-recognition receptor proteins. Pattern recognition, in turn, provokes a multitude of downstream events, including MAMP-triggered immunity (Millet et al., 2010) and the induction of early plant defence responses, which comprise the deposition of callose and the production of defence-relates secondary metabolites, e.g. phytoalexins, glucosinolates, and camalexin (Jacobs et al., 2011; Lahrmann et al., 2015). At later stages of the infection, the controlled reduction of plant defence responses becomes paramount to facilitate the establishment of the mutual interaction between the endophyte and its host plant. In this respect, balancing of plant hormone contents and the tight control of indole glucosinolates are reported to play essential roles (Nongbri et al., 2012; Lahrmann et al., 2015; Xu et al., 2018).

The elevation of cytosolic Ca^{2+} concentrations in Arabidopsis root cells through the influx of Ca^{2+} via the CYCLIC NUCLEOTIDE GATED CHANNEL 19 (CNGC19) represents a further critical asset in consolidating the plant-fungus interaction (Vadassery et al., 2009; Jogawat et al., 2020). Calcium is an essential plant macronutrient that plays an important role in plant growth and development. At the same time, Ca^{2+} serves as an important second messenger in plants that is involved in orchestrating adequate responses to external signals, including biotic stresses (Thor, 2019). Cytosolic Ca^{2+} concentrations show highly dynamic and specific spatiotemporal patterns, which are governed by the type and intensity of the perceived stimulus (Pivato and Ballottari, 2021). Depending on the particular stimulus, plant cells respond by producing specific Ca^{2+} signatures that differ in their frequency, amplitude, and duration (Batistič and Kudla, 2012). To decipher the different Ca^{2+} signatures, plants possess a broad set of different receptor molecules that either directly modify target proteins through phosphorylation or act through their physical interaction with specific partner proteins, including protein kinases (Kudla et al., 2018). This diverse set of Ca^{2+} sensor molecules encompasses Calmodulins (CaMs), Calmodulin-like proteins (CMLs), Ca^{2+}-dependent protein kinases (CDPKs), Calcineurin B-like proteins (CBLs), as well as their interacting kinases (CIPKs). The latter forming a two-component system in which the CBLs act as Ca^{2+} sensors that relay their stimulation to specific CIPKs, which subsequently interact with downstream target proteins, such as nutrient transporters or ion channels (Liu and Tsay, 2003; Maierhofer et al., 2014; Ragel et al., 2015). In particular, the regulation of the high affinity potassium transporter HAK5 and the highly selective potassium channel AKT1 by CIPK23 is well documented (Lee et al., 2007; Lan et al., 2011; Ragel et al., 2015). However, there is also evidence for an interaction of CIPKs with diverse components of the abscisic acid response pathway, including the co-repressors ABI1 and ABI2 (Ohta et al., 2003). The Arabidopsis genome contains 10 *CBL*and 26 *CIPK* genes. Among each other, the CBLs and CIPKs form functional complexes and it is noteworthy that CBLs are not promiscuously interacting with all CIPKs, but show preferences and interact only with a specific subset of CIPKs to facilitate efficient signal transduction and integration (Kudla et al., 2010). Overall, the

specific combination of CBL-CIPK modules is the key to provide versatility and flexibility in the regulation of a multitude of external stimuli that marshal ion transport in plants (Tong et al., 2021).

Up to date, the functional role of CBL7 is only partially elucidated. A recent study associates CBL7 with the regulation of plant responses towards low nitrate in Arabidopsis (Ma et al., 2015). The work highlights a substantial expression of *CBL7* in root tissues and its induction under nitrogen and nitrate limiting conditions. Moreover, the authors report on the involvement of CBL7 in the transcriptional regulation of two high-affinity nitrate transporter genes, *NRT2.4* and *NRT2.5* , without providing evidence for a molecular mechanism that could explain how the downstream genes are targeted. It is speculated that the localization of CBL7 to the nucleus facilitates its interaction with nitrate-starvation response-related transcription factors, such as NLP7 (Konishi and Yanagisawa, 2013; Marchive et al., 2013; Kiba and Krapp, 2016; Krouk and Kiba, 2020). The experimentally evidenced localization of CBL7 to the nucleus and cytoplasm (Batistič et al., 2010) is, however, inconsistent with the correlation of CBL7 with the plasma membrane localized H^+-ATPase AHA2 (Yang et al., 2019). According to this study, CBL7 inactivates AHA2 under normal conditions through the formation of a larger CBL7-CIPK11-AHA2 complex. A direct interaction between CBL7 and CIPK11 is, however, likely to be excluded, as a previous study discarded the interaction of those proteins (Fuglsang et al., 2007). In contrary, this study points towards a possible recruitment of CBL2 to the complex, because CBL2 is the only CBL that showed physical interaction with CIPK11 in the study. Under certain stress conditions, the complex dissolves and AHA2 is released from repression, which consequently translates into the efflux of H^+ from the cytoplasm.

In this study, we identified the Ca^{2+} sensor CBL7 as an essential molecular component in the interaction between the root colonizing endophyte *S. indica* and *A. thaliana* .*CBL7* is consistently induced upon root infection with the fungus, not only at early stages of the infection, but also at later phases. Furthermore, we were able to demonstrate that the availability of CBL7 is crucial for the development of the fungus-mediated plant growth promotion and for the proper distribution of potassium in the plant. The comprehensive transcriptomics analysis of the *cbl7* mutant grown with and without the fungus in comparison to corresponding wild-type plants additionally pinpointed a role of CBL7 in harmonizing plant defense responses for the long-term interaction between the endophyte and Arabidopsis. Taken together, our results establish CBL7 as a novel key component required for the successful establishment of the symbiosis between *S. indica* and *A. thaliana* .

Materials and methods

2.1. Biological material and growth conditions

In this work, we used the *Arabidopsis thaliana* Col-0 (N1092) and Col-3 (N28171) backgrounds as references. We obtained the mutant alleles*cipk13-1* (SALK_124748C) and *cipk13-2* (SALK_130671), as well as *cbl7-1* (SAIL_201_A01) from the Nottingham Arabidopsis Stock Center (NASC). T-DNA insertion lines were genotyped as previously described (Alonso et al., 2003), using the primer pairs listed in**Supplemental Data Sheet 1** . The T-DNA insertion mutants*cbl7-2* and *hak5* have previously been described (Ma et al., 2015; Ragel et al., 2015). Plants were grown on Petri dishes containing solidified $\frac{1}{2}$ MS medium supplemented with 1% sucrose (w/v) (Murashige and Skoog, 1962). Plant growth proceeded in a growth chamber under strictly controlled environmental conditions (16 h light, 8 h darkness, constant temperature of 22 ^{o}C, 100 to 105 µmol photons m^{-2} s^{-1}photosynthetically active radiation). In addition, we used*Serendipita indica* strain DSM 11827, which was obtained from the German Collection of Microorganisms and Cell Cultures (DSZM) in Braunschweig, Germany. The fungus was grown at 28 ^{o}C in darkness on solidified arginine phosphate (AP) medium (Rodríguez-Navarro and Ramos, 1984) and refreshed weekly.

2.2. Root growth promotion assay

Surface-sterilized Arabidopsis seeds were plated on vertical $\frac{1}{2}$ MS plates. After stratification (2 days at 4 ^{o}C), the plates were transferred to the growth chamber and the seedlings were grown vertically for one week. Thereafter, four to five seedlings were transferred to Petri dishes containing solidified Plant Nutrition Medium (PNM) supplemented with 50 mM NaCl (Johnson et al., 2013). Each seedling was then associated with a 5 mm Ø medium plug extracted from either sterile AP plates (control) or from AP plates harboring a one-week-old*S. indica* mycelium (co-cultivation). The PMN plates with the control seedlings and the seedlings co-cultivated with the fungus were further kept in a growth chamber maintained at 23.5 ^{o}C, 16/8 h photoperiod, 100 µmol photons m^{-2} s^{-1} light intensity for another fourteen days. After that time, the plants were photographed for the further analysis of the root system and the plant material was either used for RNA extraction or the determination of the fresh weight (fw).

2.3. Quantitative analysis of root system architecture traits

The stimulation of root growth is a well-described trait in the interaction of *S. indica* with its host plant. With the aim to quantify the effect of *S. indica* in the different genotypes and treatments, respectively, photographs of the plates were captured with a digital camera at a fixed distance of 29 cm. Using Adobe Photoshop CC, the images were cropped to a height of 14 cm maintaining only the part containing the root system and converted the pictures to black and white images. The root network traits of the plants in the prepared images were then analyzed using the GiA Roots software (Galkovskyi et al., 2012). Further processing of the images included their segmentation employing global thresholding (Binary_inverted) and Gaussian adaptive thresholding. For the comparative analysis of alterations of the root system architecture, the total network area and total network length was used as readout. Taking the biological variability of the system into account, 24 individual plants per genotype and growth condition were analyzed, respectively.

2.4. Total RNA extraction, library construction, and RNA-seq analysis

To study transcriptional alterations provoked by either the co-cultivation of Arabidopsis roots with *S. indica* or the functional knockout of *CBL7* , total RNA from 100 mg plant roots was extracted as previously described (Oñate-Sánchez and Vicente-Carbajosa, 2008). The quality and concentration of the extracted RNA was tested by absorbance analysis using a Nanodrop® ND-1000 spectrophotometer (ThermoFisher). After an additional confirmation of the RNA sample integrity on a Bioanalyzer 2100 (Agilent) by the Novogene Genomics Service (Cambridge, UK), the service laboratory proceeded with the library construction and RNA sequencing (PE150) on Illumina NovaSeq 6000 platforms. The Novogene Genomics Service also provided basic data analysis applying their RNAseq pipeline. This included data filtering and sequence alignment using HISAT2 v2.0.5 with default parameters (Kim et al., 2019), transcript quantification with HTSeq v0.6.1 with -m union parameter (Anders et al., 2014), and differential gene expression analysis employing the DESeq2 v1.22.2 algorithm with a cut-off value of an adjusted p -value of < 0.05 (Love et al., 2014). For each genotype and treatment, respectively, three biological replicates were processed. The resulting p -values were adjusted for multiple testing using the Benjamini–Hochberg correction (Benjamini and Hochberg, 1995). Along with the adjusted p -value (FDR) of < 0.05 an absolute differential expression of $\log_2$ fold change (FC) [?] 1.25 was chosen to select differentially expressed genes (DEGs). The functional classification of DEGs was performed using the MapMan v3.6 software (Thimm et al., 2004), paying special attention to DEGs related with plant defense and nutrient assimilation. Furthermore, functional relationships between the DEGs were investigated using the applications stringApp v1.7 (Doncheva et al., 2019), MCODE v2.0 (Bader and Hogue, 2003), EnrichmentMaps v3.3.3 (Merico et al., 2010), and ClueGO v2.5.8 (Bindea et al., 2009) in Cytoscape v3.9.0 (Shannon et al., 2003).

2.5. qPCR analysis

Real-time quantitative RT-PCR was conducted as previously described (Perez-Alonso et al., 2021). In brief, total RNA from three different biological samples was converted into cDNA using M-MLV reverse transcriptase and oligo$(dT)_{15}$ primer. Two nanograms of cDNA was then used as template for the qPCR reactions, which were conducted in triplicate (technical replicates). The oligonucleotide pairs used in the experiments are given in **Supplementary Data Sheet 1** . The reactions were monitored on a Lightcycler 480 Real-time PCR system (Roche Diagnostics). Differential gene expression in Arabidopsis was analyzed by using the comparative $2^{-[?][?]CT}$ method (Livak and Schmittgen, 2001) with*ADENINE PHOSPHORIBOSYL TRANSFERASE 1* (*APT1* , At1g27450) as reference gene (Jost et al., 2007). Root colonization with*S. indica* was monitored with a primer pair for fungal translation elongation factor EF-1α (*SiTEF1*) (Bütehorn et al., 2000). The fungal *SiTEF1* cDNA levels were expressed relative to the plant*GLYCERINALDEHYDE-3-PHOSPHATE DEHYDROGENASE C2* (*GAPC2* , At1g13440) cDNA levels. To exclude that the amplified DNA fragments stem from DNA of dead fungal tissues within the roots, all data presented here derived from cDNA libraries generated from RNA of colonized roots.

2.6. Trypan blue staining of fungal hyphae and spores

To visually inspect root colonization, 10-12 small root samples from control and co-colonized plants were employed. First, the root samples were thoroughly washed with deionized water. Next, the root samples were cut in 1 cm long pieces and incubated overnight in 10 N KOH. The root samples were then rinsed 5 times with sterile H_2O, before they were incubated for 5 min in 0.1 N HCl. Finally, the samples were incubated in a 0.05% trypan blue solution (w/v), before they were partially decolorized with lactophenol over ten minutes. Before the specimen were mounted on glass slides and examined by microscopy, they were washed once with 100% ethanol and thrice with sterile H_2O and stored in 60% glycerol (v/v).

2.7. Yeast two-hybrid analysis

In order to examine the physical interaction between CBL7 and CIPK13, total RNA from four weeks-old Arabidopsis seedlings (Col-0) was extracted (Oñate-Sánchez and Vicente-Carbajosa, 2008) and first-strand synthesis was performed according to the supplier's instructions, using M-MLV reverse transcriptase and oligo$(dT)_{15}$ primer (Promega). For the cloning of *CBL7* and *CIPK13* , the corresponding cDNA fragments were amplified by PCR using specific primer pairs listed in **Supplementary Data Sheet 1** . The resulting PCR fragments were inserted into the vector pGEM® -T Easy (Promega). Sequence integrity of the obtained products was confirmed by commercial sequencing (StabVida). Subsequently, the fragments were introduced into the vector pENTR-3C (Thermo Fisher) using the *Eco* RI and*Sal* I restriction sites included in the primer sequences. The obtained pENTR-CBL7 and pENTR-CIPK13 vectors were used in Gateway LR-recombination reactions with the destination vectors pDEST-22 and pDEST-32 according to the manufacturer's instructions. The resulting destination vectors were then used to transform*Saccharomyces cerevisiae* strain HF7c. Transformants were plated on SD (simple drop-out) medium /-Leu/-Trp/+Ade and incubated at 28ºC for 2 days. Protein interaction was tested by growing transformants on SD medium /-Leu/-Trp/-His/+Ade. The plates were incubated at 28ºC for up to one week. To investigate the interaction of CBL7 with two additional putative interaction partners, CIPK9 and CIPK24, the yeast two-hybrid vectors PDEST-AD092F08 and PDEST-AD107D03 for CIPK9 and CIPK24, respectively, were obtained from the Arabidopsis Biological Resource Center (ABRC).

2.8. K^+ ion content quantification

The analysis of endogenous cation contents of infected and control plants was performed in fractions of root and shoot samples. To avoid carry overs from the medium, roots were thoroughly rinsed with 10 mM MES-Ca^{2+} pH 6.5. Next, root and shoot samples were dried, weight and extracted with 1 M HNO_3. The K^+

contents of the supernatants were then determined by atomic emission spectroscopy. The results are given as the means and their standard errors of three independent experiments.

2.9. Statistical analysis

For statistical data assessment and the generation of box plots, JASP v0.16 was employed. The box plots show the median, quartiles, and extremes of the compared experimental values. One-way anova followed by Tukey's post-hoc test or Student's t -test were performed to statistically analyze the data. Sample sizes (n) for each experiment are given in the respective figure legends. Hierarchical clustering and heatmaps of selected gene expression levels across the different experiments was conducted using the Instant Clue software v0.10.10 (Nolte et al., 2018).

Results

3.1. *CBL7* is induced in the symbioses between Arabidopsis and *S. indica*

The growth promoting effect of *S. indica* on multiple host plant species has already been well-characterized (Mensah et al., 2020). However, the precise molecular mechanism by which the fungal root endophyte induces plant growth remains largely elusive. To shed further light on the molecular basis of the interaction between *S. indica*and Arabidopsis, we took a comprehensive transcriptomics approach analyzing the transcriptional differences between control plants and plants challenged with *S. indica* at both early (2 days post infection (dpi)) and later (10 dpi) stages of co-cultivation. At the indicated time points, total RNA was isolated and, after library construction, subjected to mRNA-seq analysis. The application of a Benjamini-Hochberg false discovery rate (FDR) ($p_{adj.}$ [?] 0.05) and setting the fold-change cut-off to $\log_2$FC [?] 1.25, identified 138 induced and 10 repressed genes, respectively, at 2 dpi, while we identified 411 induced and 26 repressed genes after ten days of co-cultivation (**Supplemental Data Sheet 2**). The expression data revealed that 9.8% of the differentially expressed genes (DEGs) were induced both after two days and ten days of co-cultivation, while 16.2% and 67.3% of the DEGs were only induced in the early and later phases of the interaction, respectively (**Figure 1A**). Notably, only 6.8% of the DEGs were repressed under the tested conditions, and only one gene,*SENESCENCE-ASSOCIATED GENE 13* (*SAG13*), turned out to be repressed during the establishment of the plant-fungus interaction, while being induced at later stages of the symbiosis. To gain further insight into the affected biological processes triggered by the fungus during the studied interaction, we performed gene ontology (GO) analyses of the induced DEG groups (**Supplemental Image 1A**). The analysis revealed a substantial enrichment of genes associated with stress and defense-related GO terms. To decipher possible molecular mechanisms, we further employed a functionally enriched network analysis to identify biological interpretations and interrelations of functional groups in biological networks (**Figure 1B**) (Bindea et al., 2009). The network analysis largely confirmed the obtained enriched GO term classifications. In addition, it provided evidence for the enrichment of genes related to a group of GO terms associated with cellular Ca^{2+} homeostasis and Ca^{2+} signaling processes, which attracted our attention and on which we followed up in this study.

To gain a better understanding of the role of Ca^{2+}-related processes in the mutual interaction between *S. indica* and Arabidopsis, we extracted the relative expression profiles of 88 calcium signaling-related genes from the non-filtered RNA-seq datasets and performed a hierarchical cluster analysis (**Supplemental Image 1B**). We have been particularly intrigued by the expression profile of the genes in Cluster 1, which contained the four genes *HIGH AFFINITY K^+ TRANSPORTER 5* (*HAK5*), *CALMODULIN-LIKE 4* (*CML4*),*CBL-INTERACTING PROTEIN KINASE 13* (*CIPK13*), and*CALCINEURIN B-LIKE PROTEIN 7* (*CBL7*). The genes were consistently induced both at early and later stages of the plant-fungus interaction, which could be confirmed by qRT-PCR analysis (**Figure 1C**). Activation of these genes might therefore be required both for the establishment and preservation of the mutual plant-fungus interaction.

3.2. CBL7 is required for fungus-mediated growth promotion

CBL proteins are plant-specific Ca^{2+} sensors that decode transient calcium fluctuations through the interaction with CBL-interacting protein kinases (CIPKs) (Tang et al., 2020). It has previously been shown that several CBL proteins, i.e. CBL1, CBL8, CBL9, and CBL10, interact with CIPK23 to regulate the transport activity of HAK5 (Ragel et al., 2015). Hence, we speculate that CBL7 and CIPK13 may physically interact with each other to control HAK5 activity as their downstream target, because of their shared expression profiles in response to the infection of Arabidopsis seedlings with*S. indica* . To test this hypothesis, we first conducted a yeast two-hybrid analysis (**Figure 2**). The corresponding full-length sequences of CBL7 and CIPK13 were included into the pDEST-22/pDEST-32 yeast 2-hybrid system vectors. The direct interaction of the two proteins was monitored by the ability of transformed *Saccharomyces cerevisiae* HF7c cells to grow on selection medium (SD/-Trp/-Leu/-His) containing 5 mM 3-amino-1,2,4-triazole. **Figure 2A** provides evidence of a weak interaction between CBL7 and the protein kinase CIPK13. A GeneMANIA protein-protein interaction query (Warde-Farley et al., 2010), provided additional evidence for the direct interaction of CBL7 with two other CIPK proteins (**Figure 2B**), CIPK9 and CIPK24. However, we were not able to verify the predicted interaction of CBL7 with these two CIPKs by yeast two-hybrid studies (**Supplemental Image 2**).

Next, we took a reverse genetics approach to assess the role of CBL7, CIPK13, and HAK5 in the fungus-mediated promotion of plant growth. To do so, we used two independent T-DNA insertion mutants for both *CBL7*and *CIPK13* , as well as the previously described *hak5*mutant. The mutants were grown for seven days on 1/2 MS medium alongside with corresponding wild-type control plants, before the seedlings were transferred to PNM medium, where they were either co-cultivated with*S. indica* or a mock control. After ten days, the vertically grown plants were photographed, and the root system architecture was analyzed using the GiA Roots software. The statistical assessment of the monitored parameters facilitated a quantitative comparison of the growth promoting effect elicited by *S. indica* in the different genotypes. As shown in **Figure 3A** , the functional knockout of*CIPK13* had no impact on the total root network length. Both*cipk13* alleles exhibited a significant growth promotion through the co-cultivation with *S. indica* , similar to the response of the wild-type control plants. The same observation was made for the*hak5* mutant (**Figure 3C**). In contrast, the loss-of-function mutants of *CBL7* , *cbl7-1* and*cbl7-2* , are characterized by a substantial reduction of the growth promoting effect observed in Col-3 control plants (**Figure 3B**). The additional analysis of the total network area of the different groups of seedlings confirmed the obtained results (**Table 1**). Taken together, the results suggest that CBL7 plays an important role in the establishment/maintenance of the symbiosis, because one the one hand the functional inactivation of CBL7 coincides with a loss of the growth promoting effect normally exerted by the fungus. CIPK13 and HAK5, on the other hand, are seemingly likely not vital or replaceable in the plant-fungus interaction, given that the knockout mutants showed no significant alteration of their growth behavior when infected with*S. indica* .

3.3. A loss of *CBL7* interferes with potassium distribution in Arabidopsis

To further investigate the biological functions of CBL7, we conducted another set of RNA-seq analyses comparing the transcriptional profiles of *cbl7-2* and Col-3 (wt) plants under control conditions. After applying the same threshold as before (FDR $p_{adj.}$ [?] 0.05, log_2FC [?] 1.25), we identified only 20 induced and 53 repressed DEGs (**Figure 4A,B**), respectively. The GO analysis of this reduced number of DEGs did not provide evidence for the significant enrichment of any GO term. As the arbitrary chosen expression threshold provided no significant results, we repeated the analysis with less stringent threshold values (FDR $p_{adj.}$ [?] 0.05, log_2FC [?] 0.35), giving 119 induced and 947 repressed DEGs, respectively (**Figure 4A** ,**B**). Subsequent GO and KEGG enrichment analysis revealed the repression of 33 genes associated with the spliceosome (**Figure 4C, Supplementary Data Sheet 3**). Furthermore, we were able to identify the induction of 23 ($p_{adj.}$ = 1*13^{-12}) and 11 ($p_{adj.}$ = 3.45*10^{-2}) genes related to cell wall organization and ion transport GO terms, respectively (**Supplemental Data Sheet 3**). Among the latter genes, we found the high-affinity nitrate transporter *NRT2.4* as well as the low-affinity nitrate transporter *NPF2.9* . The

involvement of CBL7 in the transcriptional regulation of nitrate transporters has previously been reported (Ma et al., 2015). Additionally, we again found that the high-affinity potassium transporter gene *HAK5* was significantly induced ($\log_2$FC = 1.85, $p_{adj.}$ = 0.022). Although our previous results questioned the role of HAK5 in the plant-fungus interaction, the reiterated appearance of *HAK5* led us to analyze the potassium content in roots and shoots of *cbl7-2*and Col-3 to determine whether CBL7 could be involved in the regulation of potassium homeostasis in Arabidopsis. To quantify the K^+ content in Arabidopsis seedlings, mutant and wt seeds were germinated on 1/2 MS and then transferred to square plates with modified Hoagland agar medium lacking glucose and containing either no KCl or 1 mM KCl. After three days of acclimatization, the seedling roots were inoculated with *S. indica* chlamydospores and mock treated, respectively. After another week of co-cultivation, the potassium contents were quantified by atomic emission spectroscopy. As shown in**Figure 5** , the potassium content of the *cbl7-2* roots grown at 0 mM KCl was similar to that of the Col-3 control plants. However, the previously described decrease in K^+contents in response to the inoculation with *S. indica* (Conchillo et al., 2021) was only observed in control plants, but not in*cbl7-2* . Interestingly, the K^+ level in the shoots of *cbl7-2* plants was significantly lower than in the mock-treated wild type, equal to the level observed for Col-3 treated with the fungus. Although K^+ levels showed a tendency to decrease in *cbl7-2* shoots, there was no significant difference between mock and fungus-treated *cbl7-2* plants. Plants grown on plates containing 1 mM KCl showed a similar picture (**Figure 5B**), only wild-type Col-3 plants showed the expected decrease in K^+ in response to the *S. indica* infection. Furthermore, we found a significant accumulation of K^+in *cbl7-2* roots, while the K^+ content in*cbl7-2* shoots appeared to be significantly reduced. We have therefore concluded that CBL7 contributes to the regulation of the distribution of K^+ in the plant. The functional loss of CBL7 is unequivocally linked to an altered K^+distribution profile, which is characterized by an accumulation of K^+ in the roots, most likely due to a lack of transport of K^+ to areal plant tissues. At the same time, our data suggest that CBL7 could also play a key role in the observed decrease in K^+ levels in plants challenged with *S. indica* , as *cbl7* mutants present a reduced reduction of K^+ when co-cultivated with*S. indica* .

3.4. The *cbl7* mutant shows increased plant defense responses

The above results highlighted the transcriptional regulation of*CBL7* in response to the infection of Arabidopsis roots with*S. indica* and its involvement in K^+partitioning in the plant. With the goal of further exploring the role of CBL7 in the establishment and maintenance of the symbiosis between Arabidopsis and *S. indica* , we compared the RNA-seq data surveyed from *cbl7-2* knockout plants and corresponding Col-3 control plants infected with *S. indica* and mock treated, respectively (**Supplementary Data Sheet 3**). Quantitative analysis of differential gene expression revealed a substantial increase in induced genes in the *cbl7* mutant relative to wt, while the number of repressed genes in *cbl7* is clearly diminished (**Figure 6A**). Furthermore, the examination of the possible logical relations between the two data sets revealed four non-overlapping and two overlapping groups for DEGs in *cbl7-2* and Col-3 (*S. indica*vs mock) (**Figure 6C**). Next, we performed a GO and functional network analysis to identify biological processes significantly affected by the loss of *CBL7* . The main alterations in the *cbl7*mutant could be associated with plant defense-related processes, especially with metabolic pathways that are involved in the biosynthesis of secondary metabolites related to plant defense (**Figure 6B,D**). The enriched genes in these GO terms included a substantial number of cytochrome P450 enzymes, such as *CYP71B3* ,*CYP71A12* , *CYP71A13* , and *CYP71B15* (PAD3), which are known to participate in glucosinolate and camalexin biosynthesis (Glawischnig, 2007; Frerigmann et al., 2016). Furthermore, we found an induction of the myrosinase genes *BGLU34* and *BGLU35* that are involved in the turnover of glucosinolates (Wittstock and Burow, 2010). The observation of an induction of plant defense-related compounds is further supported by the induction of transcription factors (TFs) *NAC042* , *WRK33* , and *WRK51* , which have been linked with the regulation of camalexin and indole glucosinolate biosynthesis (Birkenbihl et al., 2012; Saga et al., 2012; Frerigmann and Gigolashvili, 2014; Zhou et al., 2020). Furthermore, we also observed an induction of *WRKY70* , a TF involved in modulating cell wall-related defense responses (Li et al., 2017). In addition, the functional analysis of the transcriptomics data revealed an enrichment of glutathione S-transferases among the induced genes in *cbl7-2* , which included

the genes *GSTU10* , *GSTU12* , *GSTF3* ,*GSTF6* , and *GSTF7* . Glutathione S-transferases are readily induced by a wide range of stress conditions, including bacterial and fungal infections (Dixon et al., 2002; Gullner et al., 2018). Considering that all these processes are observed in the *cbl7*loss-of-function mutant, it must be concluded that CBL7 is involved in the suppression of multilayered defense responses to facilitate the establishment of *S. indica* in the root apoplast. However, other processes that appear enriched in *S. indica* challenged wt plants but not in *cbl7* , such as the induction of sucrose transporter genes and WRKY46 orchestrated abiotic stress responses, or the repression of bHLH100 and MYB72 mediated metal ion homeostasis, are likely to contribute to the significant difference in growth promotion between *cbl7* and wt triggered by the fungus.

Based on our hypothesis of an increased defense response in the*cbl7* knockout mutant, it must be expected that the roots of mutant plants are less well colonized by *S. indica* than comparable wt roots. To prove this, we analyzed root colonization in*cbl7* and Col-3 by qPCR and trypan blue staining. Indeed**,**root colonization of the *cbl7* mutant was significantly reduced (**Figure 7**). Our experiments have shown that CBL7 plays a critical role in maintaining the equilibrium between symbiotic interaction and plant defense that keeps the endophytic fungus load under control.

Discussion

Calcium signaling plays an important role in the regulation of a wide array of biological processes in eukaryotic physiology, including pathogenic and beneficial plant-microbe interactions (Vadassery and Oelmuller, 2009). Ca^{2+} signaling depends on the comprehensive interaction between membrane-located receptors that sense external signals, which then trigger transient changes in intracellular Ca^{2+} levels through the activation of Ca^{2+} permeable channels that release Ca^{2+} from internal stores, such as the endomembrane system, or transport Ca^{2+} across the plasma membrane. Within the cytoplasm, a wide array of different Ca^{2+}-binding proteins are responsible for deciphering the incoming transient Ca^{2+} signatures and translating them into appropriate molecular responses (Pirayesh et al., 2021). These Ca^{2+} sensors include calmodulins (CaMs), calmodulin-like-proteins (CMLs), Ca^{2+}-dependent protein kinases (CDPKs) and calcineurin B-like (CBL) proteins. The latter proteins generally interact with protein kinases (CIPKs) to form two-component systems. Together, these sensor systems orchestrate a multitude of downstream responses, including the control of various ion channels and transporters (Tang et al., 2020). In this study we showed that CBL7 is among the very few genes that display a consistent positive transcriptional response in Arabidopsis wild-type plants infected with the beneficial root endophyte *S. indica* , both at early and later stages of the interaction (**Figure 1**). Our studies also demonstrated that CIPK13 is a target of CBL7 in yeast two-hybrid studies (**Figure 2**), while the interaction with other CIPKs, i.e. CIPK9 and CIPK24, could not be experimentally confirmed. The physical interaction of CBL7 with CIPK13 attracted our attention because previous studies reported their expression in roots (Schliebner et al., 2008; Ma et al., 2015), and the protein-protein interaction of CIPK13 with the auxin transporter PIN5 (Jones et al., 2014). Although PIN5 is not involved in the directional cell to cell transport of auxin, but rather in maintaining the intracellular auxin homeostasis through shuttling auxin from the cytosol to the lumen of the endoplasmic reticulum (Mravec et al., 2009), this interaction could possibly represent an important link that could explain the growth promoting effect of *S. indica*on its host plants, which particularly manifests in a considerable induction of root growth (Perez-Alonso et al., 2020). In addition, the co-expression of *CBL7* , *CIPK13* , and *HAK5* prompted us to speculate that there might be a relevant connection between these components, which could regulate the uptake of potassium into host plants and, thus, their nutrition with this essential macronutrient. The activation of HAK5 through phosphorylation by CIPK23 has previously been described for Arabidopsis and tomato plants (Ragel et al., 2015; Amo et al., 2021). Hence, an interaction cascade between the three components appeared possible. However, when we analyzed the relevance of the different components with respect to their impact on the growth promoting effect on Arabidopsis roots, we had to realize that only CBL7, but neither CIPK13 nor HAK5 interfered with growth promotion (**Figure 3**). While both *cbl7* mutant alleles showed a severe loss of growth promotion, none of the other investigated mutants demonstrated significant differences to wt.

Although CIPKs and potassium transporters form bigger gene families, which opens the possibility that their loss might be compensated by other family members, it must be concluded that CIPK13 and HAK5 are not critical for the fungus-triggered plant growth promotion. The possible plastid localization of CIPK13 further argues against an interaction with CBL7 *in vivo*(Schliebner et al., 2008).

Previous work on the role of CBL7 highlighted its interaction with the*A. thaliana* PLASMA MEMBRANE PROTON ATPASE 2 (AHA2) (Yang et al., 2019). In their model, the CBL7/AHA2 complex is further stabilized by the interaction with PROTEIN KINASE SOS2-LIKE5 (PKS5), also referred to as CIPK11. The authors suggest that the Ca^{2+}-mediated dissociation of the CBL7/CIPK11/AHA2 complex under saline-alkali stress conditions translates into the activation of AHA2. Activation of AHA2, in turn, leads to hyperpolarization of the plasma membrane, which is likely to affect the transport activity of *Shaker* -like K^+ channels in the root. SKOR, an outward-rectifying K^+ channel, is reported to be crucial for the loading of K^+ into the xylem and, thus, the long-distant transport of K^+ within the plant (Gaymard et al., 1998). Moreover, SKOR is known to form heteromeric outward-rectifying K^+ channel units with a second *Shaker* -like channel, GORK (Dreyer et al., 2004). SKOR and GORK facilitate K^+ transport only when the plasma membrane is depolarized (Dreyer and Blatt, 2009). A recent publication further pinpointed the importance of the complex interplay between different nutrient transporter systems and proton pumps in nutrient cycling in plants (Dreyer, 2021). Based on these observations, it must be assumed that a loss of CBL7 will likely result in a disrupted regulation of AHA2 activity and, consequently, a hyperpolarization of the plasma membrane, which entails a reduced transport activity of SKOR and GORK. As a result, the assimilated K^+ would accumulate in the roots, and only reduced amounts of K^+ would reach the shoot. In fact, our analysis of K^+ levels in wild-type and *cbl7* mutants corroborates this hypothesis (**Figure 4**). The *cbl7* mutant plants showed a pronounced accumulation of K^+ in the roots, which we attribute to possible impairment of K^+ xylem transport. Potassium depletion in the shoot could trigger the induction of *HAK5* in *cbl7* , as multiple independent studies demonstrated that *HAK5* is induced under K^+ starvation (Ahn et al., 2004; Armengaud et al., 2004; Shin and Schachtman, 2004; Gierth et al., 2005). A recent study demonstrated the induction of *HAK5* in the host plant as a general consequence of the symbiotic interaction (Conchillo et al., 2021), which would also explain why we found *HAK5* under the consistently induced genes in wild-type plants (Figure 1). Furthermore, the latter study demonstrated that the inoculation of Arabidopsis with*S. indica* does not improve the K^+ nutrition of the host plant under K^+ limiting conditions. Intriguingly, the authors showed that root colonization is stimulated under K^+ limiting conditions, leading to the assumption that the endophyte may benefit from the relatively high cellular K^+ concentrations in plant cells or apoplast. The K^+ content in plant tissue was reported to be significantly reduced when plants were inoculated with *S. indica* . Our own work confirms this finding of reduced K^+levels in wt plants co-cultivated with the fungus. On the contrary, the reduction of K^+ levels upon infection with the fungus was largely absent in *cbl7* mutant plants, possibly because the flux of K^+ from the host plant to the fungus is hampered by inactivated voltage-gated potassium channels. However, to validate this hypothesis further studies are needed. Nevertheless, together with the widely missing *S. indica* -triggered growth promotion in inoculated *cbl7* mutants, this observation further underlined the vital role of CBL7 in the symbiotic plant-fungus interaction.

To further investigate the role of CBL7 in this context, we subjected the *cbl7-2* mutant to additional RNA-seq analyses. In a first analysis, we compared the transcriptional pattern of the *cbl7-2*mutant with that of the corresponding wild type, Col-3, under control conditions. After applying an arbitrary cut-off value of $\log_2$FC [?] 1.25, we identified only 73 DEGs that did not show enrichment of any biological process or function. Lowering the stringency of our analysis disclosed the repression of genes that are associated with the KEGG spliceosome term, including various splicing factors and the *SUPPRESSOR-OF-WHITE-APRICOT/SURP DOMAIN-CONTAINING PROTEIN* (*SWAP*) gene (Lorkovic et al., 2005). This suggests a possible impairment of alternative splicing processes in *cbl7* . It is, however, noteworthy that the gene that showed the strongest repression with a $\log_2$FC of –2.34,*MOS4-ASSOCIATED COMPLEX SUBUNIT 5C* (*MAC5C*), does not appear to form part of the spliceosome associated MAC complex (Monaghan et al., 2010), but is more closely linked with processes related with secondary cell wall synthesis (Taylor-Teeples et al., 2015). This possible relation of MAC5C with cell wall synthesis is consistent with

the observed enrichment of cell wall biogenesis-related genes, including the *HYDROXYPROLINE-RICH GLYCOPROTEIN 1* (*HRGP1*) and the proline- and leucine-rich extensin-like family protein genes*EXT3* , *EXT4* , *EXT6* , *EXT10* , and *LRX1* , respectively, which are moderately induced in *cbl7* . Extensin proteins play an important role in cell wall sensing. They are insoluble cell wall components that act as protein-protein interaction platforms to which peptide hormones and transmembrane receptors can bind, thereby relaying the perception of extracellular stimuli to the cytoplasm (Herger et al., 2019). These transcriptomic alterations possibly make the *cbl7* mutant more responsive to changes in its environment, including the perception of beneficial and pathogenic microbes on the root surface and in the apoplast.

This notion is further supported by the strongly induced plant defense response in *cbl7-2* . When comparing the transcriptional responses of *cbl7-2* plants infected with *S. indica* versus uninfected*cbl7-2* control plants with the corresponding responses of Col-3 plants, we found a significant enrichment of genes that fall into the secondary metabolite biosynthesis GO term in *cbl7-2* . This group contains the TF genes *NAC042* , *WRK33* , and *WRK51* , as well as the cytochrome P450 genes *CYP71B3* , *CYP71A12* ,*CYP71A13* , and *CYP71B15* (PAD3) encoding proteins involved in the biosynthesis of glucosinolates and camalexin, two secondary metabolites involved in the defense against pathogens (Glawischnig, 2007; Malka and Cheng, 2017). A previous study has already investigated the role of compounds derived from indole-3-acetaldoxime (IAOx) in the interaction of Arabidopsis with its root endophyte *S. indica*(Nongbri et al., 2012). The authors provide conclusive evidence that the infection of Arabidopsis with *S. indica* includes the induction of the formation of IAOx derived compounds during early stages of the interaction as a general defense response of the host plant. After the establishment of the symbiosis, the formation of indole glucosinolates and camalexin was reported to decrease. However, a certain wild-type level of these compounds appears to be required to avoid excessive root colonization, as the lack of these compounds in the IAOx-deficient *cyp79b2/cyp79b3* double mutant results in significantly increased root colonization, which is accompanied by a complete loss of fungus-conferred plant growth promotion. Excessive root colonization is suggested to convert the beneficial interaction between *S. indica*and Arabidopsis into a physiological burden for the host plant. In case of the *cbl7-2* mutant, the limitation of the formation of IAOx-derived secondary metabolites is not working properly, because we still found these plant defense-related genes induced after 14 days of co-cultivation. Consequently, it must be concluded that the mutant overreacts to the biotic stress caused by the fungus, which results in a stronger defense response and a less efficient establishment of the symbiosis. Both the visual inspection of root colonization by Trypan blue staining and the quantification of root colonization by qPCR confirmed the reduced colonization of *cbl7* mutant roots with*S. indica* relative to those of wild-type controls.

In summary, our work provides functional analyses of the cytoplasmic Ca^{2+} sensor CBL7 in the context of plant-fungus interactions. Based on the presented results, we propose that*CBL7* is induced during root infection of Arabidopsis with*S. indica* and contributes to the control of the classical plant defense. The loss of *CBL7* unequivocally blocks the normally observed promotion of *S. indica* -triggered plant growth, likely through a reduced root colonization, as the long-term harmony between the two symbionts is out of balance in the mutant. Furthermore, our work shed additional light on the so far undisclosed role of CBL7 in controlling potassium translocation in the plant body, most probably through its interaction with the plasma membrane ATPase AHA2. Under normal conditions, Ca^{2+} signals elicited by the root infection trigger the dissociation of the CBL7/CIPK11/AHA2 complex (Yang et al., 2019), which subsequently results in the hyperpolarization of the membrane. Consequently, this leads to the inactivation of outward rectifying potassium channels and a reduced flux of K^{+}into the xylem, which in turn results in an accumulation of potassium in the roots. This increase in potassium levels in the root is possibly an important asset in establishing the interaction between *S. indica*and Arabidopsis, as a very recent study demonstrated that the fungus may benefit not only from plant-derived photoassimilates, but also from the relatively high potassium contents of plant tissues (Conchillo et al., 2021). It will be a thrilling future task to decipher whether impaired repression of plant defense responses or out-of-control potassium translocation in *cbl7* are the cause of the observed reduced root colonization and missing fungus-triggered plant growth promotion.

Funding

This work was financially supported by the collaborative IPSC research project executed in the framework of the EIG CONCERT-Japan joint call on Food Crops and Biomass Production Technologies and the related national funding agencies: grant PCIN-2016–037 from the Ministry of Economy and Competitiveness (MINECO), Spain, to SP and JVC; grants 01DR17007A and 01DR17007B from the Federal Ministry of Education and Research (BMBF), Germany, to JL-M and RO, respectively; grant JPMJSC16C3 from the Japan Science and Technology Agency (JST) to HS; and grant EIG_JC1JAPAN-045 from the Centre National de la Recherche Scientifique (CNRS), France, to AK. In addition, the project obtained financial support by grant PID2020-119441RB-I00 funded by MCIN/AEI/10.13039/501100011033 and, as appropriate, by "ERDF A way of making Europe", by the "European Union" or by the "European Union NextGenerationEU/PRTR" to SP, as well as by grant REDES #190078 and Fondecyt 1211057 to PR from the Agencia Nacional de Investigacion y Desarrollo (ANID, Chile). CGG was supported by the Severo Ochoa Program for Centers of Excellence in R&D from the Agencia Estatal de Investigacion, Spain, grant SEV-2016-0672 (2017–2021) to the Centro de Biotecnologia y Genomica de Plantas.

Acknowledgments

The authors are grateful to Mar Gonzalez Ceballos for her excellent technical support. Moreover, we acknowledge the critical discussion of our work with experts in the field, including Ingo Dreyer (Universidad de Talca, Chile) and Begona Benito (CBGP), who also kindly shared mutant seeds with us. In addition, the authors thank the NASC and ABRC for providing T-DNA insertion mutant seeds and plasmids for yeast two-hybrid studies.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

SP, RO, JLM, JCV, AK and HS conceived and planned the study. MMPA, CGG, AGOV, POG, SSS and TK performed experimental procedures and collected data. MMPA, CGG, AGOV, POG, SSS, PR and SP performed analysis and SP wrote the manuscript.

Data Availability Statement

All data supporting the findings of this study are available within the paper and within its supplementary data published online.

Short legends for Supporting Information

Supplemental Data Sheet 1: Primers used for genotyping, cloning and expression

analysis

Supplemental Data Sheet 2: RNA-seq analysis of *S. indica* and mock treated Arabidopsis wild-type seedlings.

Supplementary Data Sheet 3: RNA-seq analysis of *S. indica* and mock treated Arabidopsis *cbl7-2* and Col-3 seedlings

Supplemental Image 1: GO and cluster analysis of induced DEGs in*S. indica* challenged wt plants compared to mock treated control plants.

Supplemental Image 2: Yeast two-hybrid analysis of the interaction between CBL and CIPK9 and CIPK24.

References

Ahn, S.J., Shin, R., and Schachtman, D.P. (2004). Expression of KT/KUP genes in Arabidopsis and the role of root hairs in K+ uptake.*Plant Physiol* 134(3)**,** 1135-1145. doi: 10.1104/pp.103.034660.

Alonso, J.M., Stepanova, A.N., Leisse, T.J., Kim, C.J., Chen, H., Shinn, P., et al. (2003). Genome-wide insertional mutagenesis of*Arabidopsis thaliana* . *Science* 301(5633)**,** 653-657. doi: 10.1126/science.1086391.

Amo, J., Lara, A., Martinez-Martinez, A., Martinez, V., Rubio, F., and Nieves-Cordones, M. (2021). The protein kinase SlCIPK23 boosts K^+ and Na^+ uptake in tomato plants.*Plant Cell Environ* 44(12)**,** 3589-3605. doi: 10.1111/pce.14189.

Anders, S., Pyl, P.T., and Huber, W. (2014). HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics*31(2)**,** 166-169. doi: 10.1093/bioinformatics/btu638.

Armengaud, P., Breitling, R., and Amtmann, A. (2004). The potassium-dependent transcriptome of Arabidopsis reveals a prominent role of jasmonic acid in nutrient signaling. *Plant Physiol*136(1)**,** 2556-2576. doi: 10.1104/pp.104.046482.

Bader, G.D., and Hogue, C.W.V. (2003). An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics* 4(1)**,** 2. doi: 10.1186/1471-2105-4-2.

Bakshi, M., Sherameti, I., Meichsner, D., Thurich, J., Varma, A., Johri, A.K., et al. (2017). *Piriformospora indica* Reprograms Gene Expression in Arabidopsis Phosphate Metabolism Mutants But Does Not Compensate for Phosphate Limitation. *Front Microbiol* 8**,**1262. doi: 10.3389/fmicb.2017.01262.

Batistič, O., and Kudla, J. (2012). Analysis of calcium signaling pathways in plants. *Biochimica et Biophysica Acta (BBA) - General Subjects* 1820(8)**,** 1283-1293. doi: 10.1016/j.bbagen.2011.10.012.

Batistič, O., Waadt, R., Steinhorst, L., Held, K., and Kudla, J. (2010). CBL-mediated targeting of CIPKs facilitates the decoding of calcium signals emanating from distinct cellular stores. *Plant J*61(2)**,** 211-222. doi: 10.1111/j.1365-313X.2009.04045.x.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing.*Journal of the Royal Statistical Society: Series B (Methodological)* 57(1)**,** 289-300. doi: 10.1111/j.2517-6161.1995.tb02031.x.

Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A., et al. (2009). ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks.*Bioinformatics* 25(8)**,** 1091-1093. doi: 10.1093/bioinformatics/btp101.

Birkenbihl, R.P., Diezel, C., and Somssich, I.E. (2012). Arabidopsis WRKY33 is a key transcriptional regulator of hormonal and metabolic responses toward *Botrytis cinerea* infection. *Plant Physiol*159(1)**,** 266-285. doi: 10.1104/pp.111.192641.

Bütehorn, B., Rhody, D., and Franken, P. (2000). Isolation and Characterisation of Pitef1 Encoding the Translation Elongation Factor EF-1α of the Root Endophyte Piriformospora indica. *Plant Biology*2(6)**,** 687-692. doi: 10.1055/s-2000-16647.

Conchillo, L.B., Haro, R., and Benito, B. (2021). K^+nutrition exchange in Serendipita-Arabidopsis symbiosis: study of the fungal K^+ transporters involved. *Front. Ecol. Evol.* 9**,** 789371. doi: 10.3389/fevo.2021.789371.

de Bary, A. (1879). "Die Erscheinung der Symbiose," in *Vortrag auf der Versammlung der Naturforscher und Artze zu Cassel* . (Strassburg: Verlag von K.J. Trübner), 1–30.

Dixon, D.P., Lapthorn, A., and Edwards, R. (2002). Plant glutathione transferases. *Genome biology* 3(3)**,**REVIEWS3004-REVIEWS3004. doi: 10.1186/gb-2002-3-3-reviews3004.

Doncheva, N.T., Morris, J.H., Gorodkin, J., and Jensen, L.J. (2019). Cytoscape StringApp: Network Analysis and Visualization of Proteomics Data. *Journal of Proteome Research* 18(2)**,** 623-632. doi: 10.1021/acs.jproteome.8b00702.

Dreyer, I. (2021). Nutrient cycling is an important mechanism for homeostasis in plant cells. *Plant Physiology* 187(4)**,**2246-2261. doi: 10.1093/plphys/kiab217.

Dreyer, I., and Blatt, M.R. (2009). What makes a gate? The ins and outs of Kv-like K+ channels in plants. *Trends Plant Sci*14(7)**,** 383-390. doi: 10.1016/j.tplants.2009.04.001.

Dreyer, I., Poree, F., Schneider, A., Mittelstadt, J., Bertl, A., Sentenac, H., et al. (2004). Assembly of plant *Shaker* -like K_{out} channels requires two distinct sites of the channel a-subunit. *Biophys J* 87(2)**,** 858-872. doi: 10.1529/biophysj.103.037671.

Frerigmann, H., and Gigolashvili, T. (2014). MYB34, MYB51, and MYB122 distinctly regulate indolic glucosinolate biosynthesis in*Arabidopsis thaliana* . *Mol Plant* 7(5)**,** 814-828. doi: 10.1093/mp/ssu004.

Frerigmann, H., Pislewska-Bednarek, M., Sánchez-Vallet, A., Molina, A., Glawischnig, E., Gigolashvili, T., et al. (2016). Regulation of Pathogen-Triggered Tryptophan Metabolism in Arabidopsis thaliana by MYB Transcription Factors and Indole Glucosinolate Conversion Products.*Mol Plant* 9(5)**,** 682-695. doi: 10.1016/j.molp.2016.01.006.

Fuglsang, A.T., Guo, Y., Cuin, T.A., Qiu, Q., Song, C., Kristiansen, K.A., et al. (2007). Arabidopsis Protein Kinase PKS5 Inhibits the Plasma Membrane H^+-ATPase by Preventing Interaction with 14-3-3 Protein. *The Plant Cell* 19(5)**,** 1617-1634. doi: 10.1105/tpc.105.035626.

Galkovskyi, T., Mileyko, Y., Bucksch, A., Moore, B., Symonova, O., Price, C.A., et al. (2012). GiA Roots: software for the high throughput analysis of plant root system architecture. *BMC Plant Biology*12(1)**,** 116. doi: 10.1186/1471-2229-12-116.

Gaymard, F., Pilot, G., Lacombe, B., Bouchez, D., Bruneau, D., Boucherez, J., et al. (1998). Identification and Disruption of a Plant Shaker-like Outward Channel Involved in K^+ Release into the Xylem Sap. *Cell* 94(5)**,** 647-655. doi: 10.1016/S0092-8674(00)81606-2.

Gierth, M., Maser, P., and Schroeder, J.I. (2005). The potassium transporter AtHAK5 functions in K^+ deprivation-induced high-affinity K^+ uptake and AKT1 K^+channel contribution to K^+ uptake kinetics in Arabidopsis roots. *Plant Physiol* 137(3)**,** 1105-1114. doi: 10.1104/pp.104.057216.

Glawischnig, E. (2007). Camalexin. *Phytochemistry* 68(4)**,**401-406. doi: 10.1016/j.phytochem.2006.12.005.

Gullner, G., Komives, T., Király, L., and Schröder, P. (2018). Glutathione S-Transferase Enzymes in Plant-Pathogen Interactions.*Frontiers in Plant Science* 9(1836). doi: 10.3389/fpls.2018.01836.

Herger, A., Dünser, K., Kleine-Vehn, J., and Ringli, C. (2019). Leucine-Rich Repeat Extensin Proteins and Their Role in Cell Wall Sensing. *Current Biology* 29(17)**,** R851-R858. doi: 10.1016/j.cub.2019.07.039.

Hertig, M., Taliaferro, W.H., and Schwartz, B. (1937). The terms symbiosis, symbiont and symbiote. *Journal of Parasitology*23**,** 326-329.

Jacobs, S., Zechmann, B., Molitor, A., Trujillo, M., Petutschnig, E., Lipka, V., et al. (2011). Broad-spectrum suppression of innate immunity is required for colonization of Arabidopsis roots by the fungus*Piriformospora indica* . *Plant Physiol* 156(2)**,**726-740. doi: 10.1104/pp.111.176446.

Jogawat, A., Meena, M.K., Kundu, A., Varma, M., and Vadassery, J. (2020). Calcium channel CNGC19 mediates basal defense signaling to regulate colonization by *Piriformospora indica* in Arabidopsis roots. *J Exp Bot* 71(9)**,** 2752-2768. doi: 10.1093/jxb/eraa028.

Jogawat, A., Vadassery, J., Verma, N., Oelmuller, R., Dua, M., Nevo, E., et al. (2016). PiHOG1, a stress regulator MAP kinase from the root endophyte fungus *Piriformospora indica* , confers salinity stress tolerance in rice plants. *Sci Rep* 6**,** 36765. doi: 10.1038/srep36765.

Johnson, J.M., Sherameti, I., Nongbri, P.L., and Oelmüller, R. (2013). "Standardized Conditions to Study Beneficial and Nonbeneficial Traits in the *Piriformospora indica* /*Arabidopsis thaliana*Interaction," in *Piriformospora indica: Sebacinales and Their Biotechnological Applications,* eds. A. Varma, G. Kost & R. Oelmüller. (Berlin, Heidelberg: Springer Berlin Heidelberg), 325-343.

Jones, A.M., Xuan, Y., Xu, M., Wang, R.S., Ho, C.H., Lalonde, S., et al. (2014). Border control-a membrane-linked interactome of Arabidopsis.*Science* 344(6185)**,** 711-716. doi: 10.1126/science.1251358.

Jost, R., Berkowitz, O., and Masle, J. (2007). Magnetic quantitative reverse transcription PCR: A high-throughput method for mRNA extraction and quantitative reverse transcription PCR. *BioTechniques*43(2)**,** 206-211. doi: 10.2144/000112534.

Kiba, T., and Krapp, A. (2016). Plant Nitrogen Acquisition Under Low Availability: Regulation of Uptake and Root Architecture. *Plant Cell Physiol* 57(4)**,** 707-714. doi: 10.1093/pcp/pcw052.

Kim, D., Paggi, J.M., Park, C., Bennett, C., and Salzberg, S.L. (2019). Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nature Biotechnology* 37(8)**,** 907-915. doi: 10.1038/s41587-019-0201-4.

Konishi, M., and Yanagisawa, S. (2013). Arabidopsis NIN-like transcription factors have a central role in nitrate signalling.*Nature Communications* 4(1)**,** 1617. doi: 10.1038/ncomms2621.

Krouk, G., and Kiba, T. (2020). Nitrogen and Phosphorus interactions in plants: from agronomic to physiological and molecular insights.*Curr Opin Plant Biol* 57**,** 104-109. doi: 10.1016/j.pbi.2020.07.002.

Kudla, J., Batistič, O., and Hashimoto, K. (2010). Calcium signals: the lead currency of plant information processing. *Plant Cell*22(3)**,** 541-563. doi: 10.1105/tpc.109.072686.

Kudla, J., Becker, D., Grill, E., Hedrich, R., Hippler, M., Kummer, U., et al. (2018). Advances and current challenges in calcium signaling.*New Phytologist* 218(2)**,** 414-431. doi: 10.1111/nph.14966.

Lahrmann, U., Strehmel, N., Langen, G., Frerigmann, H., Leson, L., Ding, Y., et al. (2015). Mutualistic root endophytism is not associated with the reduction of saprotrophic traits and requires a noncompromised plant innate immunity. *New Phytol* 207(3)**,** 841-857. doi: 10.1111/nph.13411.

Lan, W.-Z., Lee, S.-C., Che, Y.-F., Jiang, Y.-Q., and Luan, S. (2011). Mechanistic Analysis of AKT1 Regulation by the CBL–CIPK–PP2CA Interactions. *Molecular Plant* 4(3)**,** 527-536. doi: 10.1093/mp/ssr031.

Lee, S.C., Lan, W.-Z., Kim, B.-G., Li, L., Cheong, Y.H., Pandey, G.K., et al. (2007). A protein phosphorylation/dephosphorylation network regulates a plant potassium channel. *Proceedings of the National Academy of Sciences* 104(40)**,** 15959-15964. doi: 10.1073/pnas.0707912104.

Li, J., Zhong, R., and Palva, E.T. (2017). WRKY70 and its homolog WRKY54 negatively modulate the cell wall-associated defenses to necrotrophic pathogens in Arabidopsis. *PLOS ONE* 12(8)**,** e0183731. doi: 10.1371/journal.pone.0183731.

Liu, K.-H., and Tsay, Y.-F. (2003). Switching between the two action modes of the dual-affinity nitrate transporter CHL1 by phosphorylation. *The EMBO Journal* 22(5), 1005-1013. doi: 10.1093/emboj/cdg118.

Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25(4), 402-408. doi: 10.1006/meth.2001.1262.

Lorkovic, Z.J., Lehner, R., Forstner, C., and Barta, A. (2005). Evolutionary conservation of minor U12-type spliceosome between plants and humans. *RNA* 11(7), 1095-1107. doi: 10.1261/rna.2440305.

Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15(12), 550. doi: 10.1186/s13059-014-0550-8.

Ma, Q., Tang, R.-J., Zheng, X.-J., Wang, S.-M., and Luan, S. (2015). The calcium sensor CBL7 modulates plant responses to low nitrate in*Arabidopsis* . *Biochemical and Biophysical Research Communications* 468(1), 59-65. doi: 10.1016/j.bbrc.2015.10.164.

Maierhofer, T., Diekmann, M., Offenborn, J.N., Lind, C., Bauer, H., Hashimoto, K., et al. (2014). Site- and kinase-specific phosphorylation-mediated activation of SLAC1, a guard cell anion channel stimulated by abscisic acid. *Sci Signal* 7(342), ra86. doi: 10.1126/scisignal.2005703.

Malka, S.K., and Cheng, Y. (2017). Possible Interactions between the Biosynthetic Pathways of Indole Glucosinolate and Auxin. *Frontiers in Plant Science* 8(2131). doi: 10.3389/fpls.2017.02131.

Marchive, C., Roudier, F., Castaings, L., Brehaut, V., Blondet, E., Colot, V., et al. (2013). Nuclear retention of the transcription factor NLP7 orchestrates the early response to nitrate in plants. *Nat Commun* 4, 1713. doi: 10.1038/ncomms2650.

Mensah, R.A., Li, D., Liu, F., Tian, N., Sun, X., Hao, X., et al. (2020). Versatile *Piriformospora indica* and Its Potential Applications in Horticultural Crops. *Horticultural Plant Journal*6(2), 111-121. doi: 10.1016/j.hpj.2020.01.002.

Merico, D., Isserlin, R., Stueker, O., Emili, A., and Bader, G.D. (2010). Enrichment Map: A Network-Based Method for Gene Set Enrichment Visualization and Interpretation. *PLOS ONE* 5(11),e13984. doi: 10.1371/journal.pone.0013984.

Millet, Y.A., Danna, C.H., Clay, N.K., Songnuan, W., Simon, M.D., Werck-Reichhart, D., et al. (2010). Innate immune responses activated in Arabidopsis roots by microbe-associated molecular patterns. *Plant Cell* 22(3), 973-990. doi: 10.1105/tpc.109.069658.

Monaghan, J., Xu, F., Xu, S., Zhang, Y., and Li, X. (2010). Two Putative RNA-Binding Proteins Function with Unequal Genetic Redundancy in the MOS4-Associated Complex *Plant Physiology* 154(4),1783-1793. doi: 10.1104/pp.110.158931.

Mravec, J., Skůpa, P., Bailly, A., Hoyerová, K., Křeček, P., Bielach, A., et al. (2009). Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. *Nature*459(7250), 1136-1140. doi: 10.1038/nature08066.

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum* 15(3), 473-497. doi: 10.1111/j.1399-3054.1962.tb08052.x.

Nolte, H., MacVicar, T.D., Tellkamp, F., and Krüger, M. (2018). Instant Clue: A Software Suite for Interactive Data Visualization and Analysis.*Scientific Reports* 8(1), 12648. doi: 10.1038/s41598-018-31154-6.

Nongbri, P.L., Johnson, J.M., Sherameti, I., Glawischnig, E., Halkier, B.A., and Oelmuller, R. (2012). Indole-3-acetaldoxime-derived compounds restrict root colonization in the beneficial interaction between Arabidopsis roots and the endophyte Piriformospora indica. *Mol Plant Microbe Interact* 25(9), 1186-1197. doi: 10.1094/MPMI-03-12-0071-R.

Ohta, M., Guo, Y., Halfter, U., and Zhu, J.K. (2003). A novel domain in the protein kinase SOS2 mediates interaction with the protein phosphatase 2C ABI2. *Proc Natl Acad Sci U S A* 100(20),11771-11776. doi: 10.1073/pnas.2034853100.

Oñate-Sánchez, L., and Vicente-Carbajosa, J. (2008). DNA-free RNA isolation protocols for *Arabidopsis thaliana* , including seeds and siliques. *BMC Res Notes* 1, 93. doi: 10.1186/1756-0500-1-93.

Pérez-Alonso, M.-M., Guerrero-Galán, C., Scholz, S.S., Kiba, T., Sakakibara, H., Ludwig-Müller, J., et al. (2020). Harnessing symbiotic plant–fungus interactions to unleash hidden forces from extreme plant ecosystems. *Journal of Experimental Botany* 71(13),3865-3877. doi: 10.1093/jxb/eraa040.

Pérez-Alonso, M.M., Ortiz-García, P., Moya-Cuevas, J., Lehmann, T., Sánchez-Parra, B., Björk, R.G., et al. (2021). Endogenous indole-3-acetamide levels contribute to the crosstalk between auxin and abscisic acid, and trigger plant stress responses in *Arabidopsis thaliana* . *J Exp Bot* 72(2), 459-475. doi: 10.1093/jxb/eraa485.

Peškan-Berghöfer, T., Shahollari, B., Giong, P.H., Hehl, S., Markert, C., Blanke, V., et al. (2004). Association of *Piriformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant–microbe interactions and involves early plant protein modifications in the endoplasmic reticulum and at the plasma membrane. *Physiologia Plantarum* 122(4), 465-477. doi: 10.1111/j.1399-3054.2004.00424.x.

Pirayesh, N., Giridhar, M., Ben Khedher, A., Vothknecht, U.C., and Chigri, F. (2021). Organellar calcium signaling in plants: An update.*Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*1868(4), 118948. doi: 10.1016/j.bbamcr.2021.118948.

Pivato, M., and Ballottari, M. (2021). *Chlamydomonas reinhardtii*cellular compartments and their contribution to intracellular calcium signalling. *J Exp Bot* 72(15), 5312-5335. doi: 10.1093/jxb/erab212.

Prasad, D., Verma, N., Bakshi, M., Narayan, O.P., Singh, A.K., Dua, M., et al. (2018). Functional Characterization of a Magnesium Transporter of Root Endophytic Fungus *Piriformospora indica* . *Front Microbiol* 9, 3231. doi: 10.3389/fmicb.2018.03231.

Ragel, P., Ródenas, R., García-Martín, E., Andrés, Z., Villalta, I., Nieves-Cordones, M., et al. (2015). The CBL-Interacting Protein Kinase CIPK23 Regulates HAK5-Mediated High-Affinity K+ Uptake in Arabidopsis Roots *Plant Physiology* 169(4), 2863-2873. doi: 10.1104/pp.15.01401.

Rodriguez, R.J., Redman, R.S., and Henson, J.M. (2004). The Role of Fungal Symbioses in the Adaptation of Plants to High Stress Environments. *Mitigation and Adaptation Strategies for Global Change* 9(3), 261-272. doi: 10.1023/b:Miti.0000029922.31110.97.

Rodríguez-Navarro, A., and Ramos, J. (1984). Dual system for potassium transport in *Saccharomyces cerevisiae* . *Journal of bacteriology* 159(3), 940-945. doi: 10.1128/jb.159.3.940-945.1984.

Saga, H., Ogawa, T., Kai, K., Suzuki, H., Ogata, Y., Sakurai, N., et al. (2012). Identification and Characterization of ANAC042, a Transcription Factor Family Gene Involved in the Regulation of Camalexin Biosynthesis in Arabidopsis. *Molecular Plant-Microbe Interactions®*25(5), 684-696. doi: 10.1094/mpmi-09-11-0244.

Schliebner, I., Pribil, M., Zuhlke, J., Dietzmann, A., and Leister, D. (2008). A Survey of Chloroplast Protein Kinases and Phosphatases in Arabidopsis thaliana. *Curr Genomics* 9(3), 184-190. doi: 10.2174/138920208784340740.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., et al. (2003). Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Research*13(11), 2498-2504. doi: 10.1101/gr.1239303.

Shin, R., and Schachtman, D.P. (2004). Hydrogen peroxide mediates plant root cell response to nutrient deprivation. *Proc Natl Acad Sci U S A* 101(23), 8827-8832. doi: 10.1073/pnas.0401707101.

Sun, C., Shao, Y., Vahabi, K., Lu, J., Bhattacharya, S., Dong, S., et al. (2014). The beneficial fungus *Piriformospora indica* protects Arabidopsis from *Verticillium dahliae* infection by downregulation plant defense responses. *BMC Plant Biol* 14**,** 268. doi: 10.1186/s12870-014-0268-5.

Tang, R.-J., Wang, C., Li, K., and Luan, S. (2020). The CBL–CIPK Calcium Signaling Network: Unified Paradigm from 20 Years of Discoveries. *Trends in Plant Science* 25(6)**,** 604-617. doi: 10.1016/j.tplants.2020.01.009.

Taylor-Teeples, M., Lin, L., de Lucas, M., Turco, G., Toal, T.W., Gaudinier, A., et al. (2015). An Arabidopsis gene regulatory network for secondary cell wall synthesis. *Nature* 517(7536)**,**571-575. doi: 10.1038/nature14099.

Thimm, O., Bläsing, O., Gibon, Y., Nagel, A., Meyer, S., Krüger, P., et al. (2004). MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes.*Plant Journal* 37(6)**,** 914-939. doi: 10.1111/j.1365-313X.2004.02016.x.

Thor, K. (2019). Calcium—Nutrient and Messenger. *Frontiers in Plant Science* 10(440). doi: 10.3389/fpls.2019.00440.

Tong, T., Li, Q., Jiang, W., Chen, G., Xue, D., Deng, F., et al. (2021). Molecular Evolution of Calcium Signaling and Transport in Plant Adaptation to Abiotic Stress. *International Journal of Molecular Sciences* 22(22)**,** 12308.

Vadassery, J., and Oelmüller, R. (2009). Calcium signaling in pathogenic and beneficial plant microbe interactions: what can we learn from the interaction between *Piriformospora indica* and *Arabidopsis thaliana* . *Plant Signaling & Behavior* 4(11)**,** 1024-1027. doi: 10.4161/psb.4.11.9800.

Vadassery, J., Ranf, S., Drzewiecki, C., Mithöfer, A., Mazars, C., Scheel, D., et al. (2009). A cell wall extract from the endophytic fungus *Piriformospora indica* promotes growth of Arabidopsis seedlings and induces intracellular calcium elevation in roots.*Plant J* 59(2)**,** 193-206. doi: 10.1111/j.1365-313X.2009.03867.x.

Varma, A., Savita, V., Sudha, Sahay, N., Butehorn, B., and Franken, P. (1999). *Piriformospora indica,* a cultivable plant-growth-promoting root endophyte. *Appl Environ Microbiol*65(6)**,** 2741-2744. doi: 10.1128/AEM.65.6.2741-2744.1999.

Verma, S., Varma, A., Rexer, K.-H., Hassel, A., Kost, G., Sarbhoy, A., et al. (1998). *Piriformospora indica* , gen. et sp. nov., a new root-colonizing fungus. *Mycologia* 90(5)**,** 896-903. doi: 10.1080/00275514.1998.12026983.

Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., et al. (2005). The endophytic fungus *Piriformospora indica*reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proc Natl Acad Sci U S A* 102(38)**,**13386-13391. doi: 10.1073/pnas.0504423102.

Warde-Farley, D., Donaldson, S.L., Comes, O., Zuberi, K., Badrawi, R., Chao, P., et al. (2010). The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res* 38(Web Server issue)**,**W214-220. doi: 10.1093/nar/gkq537.

Weiss, M., Waller, F., Zuccaro, A., and Selosse, M.A. (2016). Sebacinales - one thousand and one interactions with land plants.*New Phytol* 211(1)**,** 20-40. doi: 10.1111/nph.13977.

Wittstock, U., and Burow, M. (2010). Glucosinolate breakdown in Arabidopsis: mechanism, regulation and biological significance.*The Arabidopsis Book* 8**,** e0134-e0134. doi: 10.1199/tab.0134.

Xu, L., Wu, C., Oelmüller, R., and Zhang, W. (2018). Role of Phytohormones in *Piriformospora indica* -Induced Growth Promotion and Stress Tolerance in Plants: More Questions Than Answers. *Front Microbiol* 9**,** 1646. doi: 10.3389/fmicb.2018.01646.

Yang, Y., Wu, Y., Ma, L., Yang, Z., Dong, Q., Li, Q., et al. (2019). The Ca^{2+} Sensor SCaBP3/CBL7 Modulates Plasma Membrane H^{+}-ATPase Activity and Promotes Alkali Tolerance in Arabidopsis. *Plant Cell* 31(6)**,** 1367-1384. doi: 10.1105/tpc.18.00568.

Zhou, J., Wang, X., He, Y., Sang, T., Wang, P., Dai, S., et al. (2020). Differential Phosphorylation of the Transcription Factor WRKY33 by the Protein Kinases CPK5/CPK6 and MPK3/MPK6 Cooperatively Regulates Camalexin Biosynthesis in Arabidopsis. *The Plant Cell*32(8)**,** 2621-2638. doi: 10.1105/tpc.19.00971.

Tables

Table 1. Growth promoting effects on Arabidopsis plants mediated by the endophytic fungus. Differences in total root network area were used as molecular marker of the growth promoting effect of*S. indica* on its host plants. Data represent means ± SE (n = 24). Asterisks indicate significant differences between *S. indica* and mock treated samples. Student's *t* -test: **P[?]0.01, ***P[?]0.001.

Genotype	Network area (cm^2)	Network area (cm^2)	Network area (cm^2)
	+ *S. indica*	– *S. indica*	[?] +/– *S. indica*
Col-3	7.31 ± 0.27	4.01 ± 0.28	3.30 ± 0.39***
cbl7-1	6.01 ± 0.29	4.97 ± 0.35	1.04 ± 0.45
cbl7-2	5.42 ± 0.26	4.41 ± 0.22	1.01 ± 0.34
Col-0	3.75 ± 0.24	2.58 ± 0.22	1.16 ± 0.33**
cipk13-1	4.31 ± 0.25	2.99 ± 0.15	1.32 ± 0.29***
cipk13-2	3.31 ± 0.14	2.24 ± 0.15	1.07 ± 0.21***
hak-5	4.65 ± 0.31	2.46 ± 0.24	2.19 ± 0.39***

Figure Legends

Figure 1. Transcriptional analysis of Arabidopsis seedlings co-cultivated with *S. indica*. **A**) Venn diagram showing the numbers of differentially expressed genes in Arabidopsis plants 2- and 14-days post infection with *S. indica* compared to control plants that were mock infected. **B**) ClueGo analysis of induced DEGs. The figure shows the representative molecular function interaction among the targets. **C**) qPCR analysis of transcriptional responses of identified target genes in plants that were co-cultivated for 2 and 14 days, respectively, with *S. indica* compared to mock treated plants. The data represent means ± SE (n = 3).

Figure 2. Protein-protein interaction (PPI) studies to identify binding partners of CBL7. A) Yeast cells co-expressing the indicated combinations of constructs were grown on non-selective (-LW) or selective (–LWH) media. Colony growth on the selective media indicated protein–protein interactions. The empty activation domain (AD) vector was used as a negative control. Fivefold diluted cell cultures were inoculated on each spot as indicated by the triangle widths. A representative data set of an experiment replicated three times on different biological samples is shown. BD, binding domain. **B**) Interaction network for CBL7 inferred from 58 publicly available datasets included in GeneMANIA v3.5.2. The PPI network indicates the protein set that either directly (diamonds) or indirectly (circles) interact with CBL7 (triangle). The different shape size refers to the calculated interaction score, while the thickness of the edges indicates the normalized maximal weight of the interaction.

Figure 3. Total root network length of Arabidopsis wild-type and mutant plants after 14 days of co-cultivation with *S. indica* or mock treatment . **A**) Comparison of two *cipk13* T-DNA insertion mutant alleles with Col-0. **B**) Analysis of the fungus-triggered growth promoting effect in Col-3 and *cbl7* mutant plants. **C**) The growth promoting effect of *S. indica* is not affected in the *hak5* mutant. The box plots show the median, quartiles, and extremes of the compared datasets (n = 24). Asterisks indicate significant differences between S. indica and mock treated samples. Student's *t* -test: **P[?]0.01, ***P[?]0.001.

Figure 4. Analysis of transcriptional differences between*cbl7* mutant and wild-type Col-3 plants. A) DEGs statistics for the *cbl7* mutant compared to Col-3 control plants under control conditions. **B**) Volcano plot of the distribution of all DEGs, mapping the 20 upregulated genes (red) and 53 downregulated genes (blue). The figure shows the relation between the significance (-$\log_{10}$(q-value)) and the strength ($\log_2$FC) of the differential expression. **C**) Bar plot of the KEGG enrichment analysis of induced DEGs in *cbl7*compared to Col-3 control plants under control conditions. Only the spliceosome KEGG term appeared to contain significantly enriched genes. Gene enrichment is given by -$\log_{10}$(q-value).

Figure 5. K^+ contents of root and shoot tissues of *S. indica* infected and non-infected control plants . The plants were grown in modified Hoagland medium under K^+ starvation (0 mM KCl) (**A**) or at 1 mM KCl (**B**). The box plots show the median, quartiles, and extremes of the compared datasets (n = 15). Different letters indicate significant differences between the compared conditions and genotypes analyzed by ANOVA and a Tukey-Kramer post hoc test ($P<0.05$).

Figure 6. Transcriptomics analysis of mock and *S. indica*treated the *cbl7* mutant plants to wild-type Arabidopsis.A) DEGs statistics for the *cbl7* mutant and Col-3 control plants after co-cultivation with *S. indica* for 14 days.**B**) KEGG enrichment analysis of induced DEGs in *cbl7*co-cultivated with the fungus versus similarly treated Col-3 plants. Each circle in the figure represents a distinct KEGG pathway, and the circle size indicates the number of genes enriched in the corresponding metabolic pathway. The significance of the observed gene enrichment is represented by a color gradient referring to the adjusted p-value (padj). **C**) Venn diagram showing the numbers of DEGs in Col-3 and *cbl7* 14-days post infection with *S. indica* compared to control plants that were mock infected. **D**) Functional network analysis of DEGs in *cbl7* and Col-3. Up- and down-regulated genes are differentiated by color and shape. The shape size refers to the different levels of differential expression according to the*log* $_2$FC levels observed in the RNAseq analysis. Functional associations are highlighted in the figure.

Figure 7. Analysis of the colonization of Col-3 and *cbl7*roots with *S. indica* . **A**) Trypan blue stain of root segments at 1 to 2 cm distance from the root tip. The figure shows representative pictures for the three studied genotypes after 14 days of co-cultivation with S. indica. B) Quantitative assessment of root colonization by qPCR analysis of *SiTEF1* . Depicted are means +- SE of three independent biological experiments performed in triplicate. The Arabidopsis *GAPC2* gene served as endogenous control. Asterisks indicate significant differences (Student's *t* -test: ***P[?]0.001).