



REVIEW PAPER

Harnessing symbiotic plant–fungus interactions to unleash hidden forces from extreme plant ecosystems

Marta-Marina Pérez-Alonso¹, Carmen Guerrero-Galán¹, Sandra S. Scholz², Takatoshi Kiba^{3,4}, Hitoshi Sakakibara^{3,4}, Jutta Ludwig-Müller⁵, Anne Krapp⁶, Ralf Oelmüller², Jesús Vicente-Carbajosa^{1,7} and Stephan Pollmann^{1,7,*}

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

³ RIKEN Center for Sustainable Resource Science, 1-7-22, Suehiro, Tsurumi, Yokohama, 230-0045, Japan

⁴ Department of Applied Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601, Japan

⁵ Institute of Botany, Technische Universität Dresden, 01062 Dresden, Germany

⁶ Institut Jean-Pierre Bourgin, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78000 Versailles, France

⁷ 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@upm.es

Received 5 November 2019; Editorial decision 15 January 2020; Accepted 21 January 2020

Editor: Peter Doerner, University of Edinburgh, UK

Abstract

Global climate change is arguably one of the biggest threats of modern times and has already led to a wide range of impacts on the environment, economy, and society. Owing to past emissions and climate system inertia, global climate change is predicted to continue for decades even if anthropogenic greenhouse gas emissions were to stop immediately. In many regions, such as central Europe and the Mediterranean region, the temperature is likely to rise by 2–5 °C and annual precipitation is predicted to decrease. Expected heat and drought periods followed by floods, and unpredictable growing seasons, are predicted to have detrimental effects on agricultural production systems, causing immense economic losses and food supply problems. To mitigate the risks of climate change, agricultural innovations counteracting these effects need to be embraced and accelerated. To achieve maximum improvement, the required agricultural innovations should not focus only on crops but rather pursue a holistic approach including the entire ecosystem. Over millions of years, plants have evolved in close association with other organisms, particularly soil microbes that have shaped their evolution and contemporary ecology. Many studies have already highlighted beneficial interactions among plants and the communities of microorganisms with which they coexist. Questions arising from these discoveries are whether it will be possible to decipher a common molecular pattern and the underlying biochemical framework of interspecies communication, and whether such knowledge can be used to improve agricultural performance under environmental stress conditions. In this review, we summarize the current knowledge of plant interactions with fungal endosymbionts found in extreme ecosystems. Special attention will be paid to the interaction of plants with the symbiotic root-colonizing endophytic fungus *Serendipita indica*, which has been developed as a model system for beneficial plant–fungus interactions.

Keywords: Abiotic stress, crosstalk, endosymbiosis, plant performance, *Serendipita indica*.

Introduction

Agriculture is the principal means of livelihood in many regions of the world. However, current agricultural practice is not sustainable and is known to cause serious ecological damage, such as soil erosion, eutrophication of water bodies, increasing salinity of soils, and desertification, which in the long run translates into substantially reduced agricultural productivity owing to further loss of arable land. Global climate change represents an additional hazard that aggravates the negative effects of current agricultural practices and causes further dramatic losses in agricultural production.

Given the increasing demand for agricultural products resulting from the growing world population, which is predicted to reach 9.5 billion people by 2050 (ISAAA, 2017), the combination of non-sustainable agricultural practices and climate change effects will not only entail economic losses for the agricultural industry but put the general food supply for humankind in considerable jeopardy. With respect to current estimates, 800–925 million people will be undernourished in 2020; ‘hidden hunger’ due to a lack of vitamins and minerals is estimated to be the most common form of malnutrition, affecting approximately 2 billion people (FAO *et al.*, 2015; Fedoroff, 2015). Hence, immediate measures must be taken to limit the damage already caused to the environment and to secure food availability in the future. Advanced agricultural biotechnology methods, including the so-called ‘new breeding techniques’ consisting of directed genome editing (i.e. CRISPR/Cas), are expected to be able to alleviate the effects of climate change and to ensure a more sustainable agriculture (Aglawe *et al.*, 2018). These approaches include, for example, the generation of more drought- and salt-tolerant crops, or plants with improved nitrogen use efficiency or pathogen resistance, by cloning genes from plants found in high-stress environments into stress-sensitive but highly productive species. It is anticipated that these actions will reduce the application of pesticides and mineral fertilizers and limit the need for irrigation (Gartland and Gartland, 2018). According to our estimations, such exclusively plant-focused strategies might be insufficient to solve the issue of food security entirely. Thus, in this review we would like to draw attention to the symbiosis of plants with commensal microorganisms, an often-underestimated factor that can substantially affect plant performance under unfavorable growth conditions.

Throughout the course of evolution plants have been constantly confronted with changing environmental conditions, forcing them to adapt in order to survive. These changing conditions included temperature fluctuations (Fig. 1), scarce water resources, and high UV radiation. Although it is extremely difficult to precisely determine the temporal dynamics of prehistoric climate changes, it is undeniable that climate change has accelerated in the past century (Murray, 1997; Petit *et al.*, 1999). The fact that anthropogenic greenhouse gas emissions considerably contribute to this acceleration raises the question of whether plants will be able to adapt to the imposed environmental stress conditions. It is possible that they will run out of time to develop appropriate responses to counteract the detrimental effects. However, assuming that this is not the first time that plants have faced such conditions, they may already have suitable molecular mechanisms at their disposal, generated during previous challenges, to withstand the foreseen unfavorable conditions of increased temperatures and water shortages.

The majority of plant studies focus only on plant responses toward abiotic stresses and disregard the fact that plants normally live in close association with a plethora of different microorganisms, such as bacteria, fungi, oomycetes, and protists, and that millions of years of co-evolution have led to the establishment of highly specialized ecosystems in which plants constantly interact with their surrounding communities of commensal, symbiotic, and pathogenic microorganisms. In the climate change context, symbiotic relationships are of particular interest as they are supposed to translate, or already have translated, into mutually advantageous associations that can provide important fitness improvements. The concept of a mutual coexistence between dissimilar organisms, referred to as symbiosis (from the ancient Greek *symbiōsis*, ‘living together’), was first described by Heinrich Anton de Bary (1879). Later, the terms symbiosis, symbiont, and symbiote were further defined by Hertig *et al.* (1937). Since these early observations, our insight into symbiotic associations of plants has substantially advanced to indicate that plant–microbe interactions are important to the structure, function, and health of plant communities and that symbiotic fungi contribute to—and may be even responsible for—the adaptations of plants to environmental stresses (Rodríguez *et al.*, 2004).

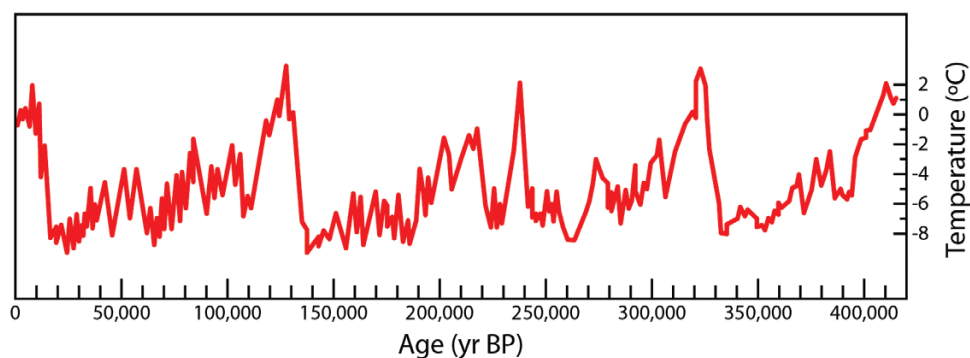


Fig. 1. Record of isotopic temperature changes of the atmosphere extracted from an Antarctic ice core (Petit *et al.*, 1999). BP, Before present.

Given that practically all plants are symbiotic with fungi, which depend either partially (endophytes) or entirely (mycorrhizae) on the interaction with their host plant, we asked ourselves the question of whether it would be potentially interesting to investigate symbiotic interactions in plant–fungal communities known to inhabit ecosystems in extreme environments. Such ‘extreme’ communities may allow us to identify patterns of crosstalk or molecular mechanisms that facilitate the creation of more sustainable agricultural systems with increased performance levels, either by harnessing identified molecular mechanisms or by soil microbiome engineering approaches. There are plenty of examples of mutualistic fungi that can confer stress tolerance upon plants. For example, a morphologically defined group of fungi within the class Ascomycota, the so-called dark septate endophytes, are well known as endosymbionts of numerous plant species, including crop plants (Jumpponen and Trappe, 1998; Andrade-Linares *et al.*, 2011). Intriguingly, Marco Molina-Montenegro and colleagues recently reported the symbiosis of two fungal endophytes, *Penicillium chrysogenum* and *Penicillium brevicompactum*, with *Colobanthus quitensis* (Caryophyllaceae) and *Deschampsia antarctica* (Poaceae), two plants that are found in the Antarctic region. It was demonstrated that the isolated root endophytes are able to increase plant performance under UV-B radiation. Moreover, the inoculation of lettuce with the isolated fungi was shown to significantly improve the ecophysiological performance and yield of the plant under both normal and drought conditions (Molina-Montenegro *et al.*, 2016; Ramos *et al.*, 2018). Another highly interesting example of an ‘extreme’ symbiosis comes from the isolation of a *Fusarium culmorum* strain that has been identified as an endophytic symbiont of dune grass (*Leymus mollis*) collected from coastal beach habitats. Remarkably, the fungal strain isolated from plants collected from this salt-stress environment was more efficient in conferring salt-stress tolerance to dune grass and tomato plants than a *F. culmorum* isolate purchased from the American Type Culture Collection; this reflects an ecological phenomenon referred to as habitat-adapted symbiosis. This habitat-specific phenomenon is suggested to provide an intergenomic epigenetic mechanism for plant adaptation and survival in high-stress environments (Rodríguez *et al.*, 2008). We made a similar observation with a *Fusarium* sp. strain isolated from sea-blite (*Suaeda maritima*) collected from saline ponds along coastal plains in India (R. Oelmüller, unpublished results).

Possibly the most extensively studied endosymbiotic fungus from an extreme environment is *Serendipita indica* (formerly named *Piriformospora indica*). *S. indica* (Agaricomycetes, Basidiomycota) is an axenically cultivable, root-colonizing endophytic fungus that was first isolated from two xerophytic woody shrubs, *Ziziphus nummularia* and *Prosopis juliflora*, in the Thar desert in India (Verma *et al.*, 1998). Closely related endophytic species have been isolated from western European and Namibian Fabaceae, Poaceae, and Araceae (Weiß *et al.*, 2011). The initially identified isolate of *S. indica* is deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany (DSM 11827). The fungus possesses exceptional ability to substantially promote plant growth and performance, especially under stress conditions,

and an outstanding versatility to colonize a very broad range of plant species (Waller *et al.*, 2005; Jacobs *et al.*, 2011; Gill *et al.*, 2016; Su *et al.*, 2017). The latter feature suggests the existence of conserved molecular mechanism(s) that facilitate promiscuous host selection, which makes *S. indica* such an interesting microorganism to study and led to a rapid suggestion that it could be applied to increase plant production (Varma *et al.*, 1999). In this review, we will provide a summary on the current knowledge of the molecular and biochemical crosstalk between *S. indica* and its hosts, and reflect on the possibilities to apply this knowledge to improve agricultural productivity.

The fungus *Serendipita indica*

Although the basidiomycete *S. indica* is classified as an endophyte, the fungus possesses many characteristics that are generally attributed to arbuscular mycorrhizal fungi (AMF), for example, its lifestyle maintaining hyphae outside the host root. Like AMF, *S. indica* is able to promote the growth of host plants and increase the resistance of colonized plants to fungal pathogens and various abiotic stresses (Harman, 2011; Abdelaziz *et al.*, 2019). Additionally, *S. indica* is known to affect the secondary metabolism of colonized host plants and to promote seed production by host plants, some of which are of economic importance (Waller *et al.*, 2005; Strehmel *et al.*, 2016). However, unlike AMF, *S. indica* can be easily cultivated (Oelmüller *et al.*, 2009) and is capable of colonizing the roots of the model dicot plant *Arabidopsis thaliana* (Peškan-Berghöfer *et al.*, 2004). In fact, *S. indica* belongs to the newly described family of Sebacinaceae and the new order Sebacinales (Agaricomycetes) (Weiss *et al.*, 2004, 2011; Qiang *et al.*, 2012a; Weiß *et al.*, 2016).

Owing to the great scientific interest in this mutualistic symbiont over the past two decades, great advances have been made with respect to the genomic assessment and manipulation of *S. indica*. As early as 2009, the group of Alga Zuccaro described a method for the stable genetic transformation of the fungus (Zuccaro *et al.*, 2009), and this was followed by a report on the deep genomic study of its 25 Mb genome (Zuccaro *et al.*, 2011). These experimental advances represent highly valuable tools for further investigation of the molecular mechanisms that allow the fungus to grow readily on diverse media and to colonize an extremely wide range of mono- and dicotyledonous plants.

Establishment of symbiosis

The genomic study additionally revealed that root colonization by *S. indica* proceeds in two steps (Zuccaro *et al.*, 2011). The interaction begins with a short biotrophic phase, which is followed by a saprophytic phase in which the fungus feeds on dead host plant cells. During the initial phase of root colonization, the host plant responds with increased programmed cell death triggered by the simultaneous induction of endoplasmic reticulum stress and repression of the unfolded protein response. Moreover, it is hypothesized that the endoplasmic reticulum stress translates into the induction of a vacuolar processing enzyme/caspase 1-like activity-dependent vacuolar

cell death program (Deshmukh *et al.*, 2006; Qiang *et al.*, 2012b). Furthermore, the host plant responds by secreting symbiosis-specific proteins, including proteins related to growth, development, biotic and abiotic stress responses, and mucilage (Thürich *et al.*, 2018). At the same time, plant innate immunity is suppressed by the manipulation of a handful of different plant hormone signaling pathways to overcome plant defenses and establish a compatible interaction between the fungus and the host (Schäfer *et al.*, 2009; Jacobs *et al.*, 2011). In particular, levels of abscisic acid (ABA), salicylic acid, and jasmonic acid, as well as jasmonoyl-L-isoleucine, are reported to substantially increase during very early phases of the interaction. The induction of stress- and defense-related hormones in the host plant can be triggered by chemical compounds released by the fungus before physical contact. It may be concluded that these transient metabolic reactions serve to prepare the plant for the symbiotic interaction. Notably, the plant response is not restricted to the root system, but spreads to aerial tissues as well. Moreover, the response is only short lived, as hormone levels return to normal levels after 6 days of co-cultivation (Vahabi *et al.*, 2015).

The exact role of phytohormones in the establishment of the symbiotic interaction is still a matter of debate (Vadassery *et al.*, 2008; Schäfer *et al.*, 2009; Lee *et al.*, 2011; Hilbert *et al.*, 2012). The broad host range of *S. indica* suggests that it evolved highly sophisticated colonization strategies, recruiting a similar or identical hormone signaling pathway from host plants in order to successfully establish a symbiotic relationship. However, recent work has cast doubts on this hypothesis of a simple reprogramming of the plant hormone-signaling machinery. A comprehensive study of the role of jasmonic acid and gibberellic acid during the colonization of seven different plant species with *S. indica* highlighted considerable differences in root colonization and plant hormone action, suggesting a high degree of species specificity in the establishment of symbiosis (Liu *et al.*, 2019).

The controversial discussion regarding the contribution of auxins to fungus-mediated growth promotion and the establishment of the mutual interaction is also noteworthy. In particular, the root growth-promoting effect of *S. indica* implied the involvement of auxins, possibly the best-characterized class of plant hormones (Fig. 2). Auxins orchestrate virtually every aspect of plant growth and development (Davies, 2010). In plant roots, changes in local auxin levels cause a number of well-described phenotypes, including a dose-dependent increase in the length of epidermal-derived root hairs, a bimodal effect on primary root elongation, and a dose-dependent increase in the number of lateral root primordia (Overvoorde *et al.*, 2010). More importantly in this context, however, is the fact that indole-3-acetic acid (IAA) and several of its precursor molecules, such as indole-pyruvic acid (IPA), indole-3-acetamide (IAM), and tryptamine (TAM), are readily synthesized by various plant-interacting fungi and appear to contribute to fungal-plant interactions (Spaepen *et al.*, 2007; Fu *et al.*, 2015). *S. indica* was shown to synthesize IAA via IPA and indole-3-acetaldehyde (IAD), while other conceivable indolic intermediates, namely IAM, TAM, and indole-3-acetonitrile, were neither detected as endogenous compounds nor converted into IAA by the fungus (Hilbert *et al.*, 2012).



Fig. 2. Growth-promoting effect of *S. indica* on young *A. thaliana* plants. The plants were grown either without *S. indica* (control, with a sterile media plug) or in the presence of a media plug that contained *S. indica* hyphae.

Further experiments provided strong evidence that mycelium-derived IAA has no significant impact on growth promotion, but rather represents a compatibility factor that is important for the establishment of the biotrophic interaction (Hilbert *et al.*, 2012). More recently, however, another report demonstrated that *S. indica* hyphae contain considerable amounts of IAM and that both IAM and IAA levels increase during the colonization of *Brassica campestris* roots (Hua *et al.*, 2017). In any case, it seems that IAA levels in host plants temporarily increase during the early phases of the interaction, only to return to the levels of non-colonized roots shortly after (Vadassery *et al.*, 2008; Hilbert *et al.*, 2012). Genome-wide expression studies and gene ontology (GO) analyses did not provide clear-cut indications of a significant enrichment of genes listed in GO terms related to auxin biosynthesis or signaling during the first 14 days after infection (Lahrmann *et al.*, 2015). Interestingly, the content of conjugated auxin increases moderately over the course of colonization in infected roots (Vadassery *et al.*, 2008). This observation may suggest that the fungus either inactivates free IAA by itself or induces the expression of *Gretchen Hagen 3 (GH3)* genes. *GH3* genes encode IAA-amidosynthases that catalyze the conjugation of free IAA to amino acids, thereby physiologically inactivating the plant hormone (Staswick *et al.*, 2005; Böttcher *et al.*, 2010, 2012). Remarkably, a recent study employing state-of-the-art live cell imaging techniques and liquid chromatography/mass spectrometry-based plant hormone analyses provided unequivocal evidence that the initial fungus-mediated increase of IAA in plant roots is seemingly adequate to induce lateral root formation within a very short timeframe (Meents *et al.*, 2019). In view of these findings, it appears tempting to speculate that the fungus-derived increase of IAA in the early phase of colonization is sufficient to trigger alterations in the developmental program of the host root,

which result in morphological changes in the root architecture, facilitating the improved nutrition of the host plant through the extended root system in the long term.

A more detailed analysis of transcriptional changes of a group of approximately 140 genes related to auxin metabolism, transport, and signaling over the first 14 days of co-cultivation with *S. indica*, however, contradicts the notion that auxin-related genes are not considerably affected. The analysis revealed the induction of a set of *GH3* genes, namely *GH3.2*, *GH3.3*, and *GH3.15*, in the infected plants, which neatly matches the observed induction of IAA–amino acid conjugates (Vadassery *et al.*, 2008). Interestingly, the *UGT84B1* gene, which codes for a UDP-glycosyltransferase that has been described to catalyze the conjugation of free IAA to glucose (Jackson *et al.*, 2001, 2002), is also significantly induced. In line with these findings, it has been observed that infection with *S. indica* is sufficient to rescue the high-auxin phenotype of the *sur1-1* mutant through the reduction of free auxin levels (Vadassery *et al.*, 2008). Together, these experiments support the hypothesis that cellular auxin levels are increased and need to be actively intercepted by conjugation with either sugar or amino acids to prevent the over-accumulation of physiologically active free IAA. The latter assumption is further strengthened by the observed induction of *PIN5* expression. *PIN5* is an auxin transporter that contributes to the maintenance of subcellular auxin homeostasis by mediating the flow of auxin from the cytoplasm into the lumen of the endoplasmic reticulum (Mravec *et al.*, 2009). Notably, two other *PIN* genes, *PIN3* and *PIN4*, also appear to be induced upon infection with *S. indica* (Fig. 3). While *PIN3*, together with auxin response factor 7 (*ARF7*), drives early steps in lateral root formation, *PIN4* is known to be involved in the generation of auxin gradients and auxin canalization in root tips (Friml *et al.*, 2002; Chen *et al.*, 2015b; Laskowski and ten Tusscher, 2017). Thus, it is suggested that they are involved in triggering the root growth-promoting effect observed in *S. indica*-infected plants. A detailed study of the role of *PIN3* and *PIN4* in the infection process is currently under way in our laboratories.

Most notable, however, is the increased flux of metabolites into the production of the defense compounds camalexin and glucobrassicin. As can be seen in Fig. 3, a large proportion of the genes encoding relevant enzymes of the two corresponding biosynthetic pathways (Fig. 4) are significantly induced upon infection with *S. indica*. The biosynthesis of camalexin and glucobrassicin could be essential to limit colonization of the host plant with *S. indica*, thus allowing the beneficial interaction while preventing over-colonization (Nongbri *et al.*, 2012). The corresponding metabolic pathways are orchestrated by a small number of transcription factors, namely *MYB34*, *MYB51*, *MYB122*, and *WRKY33* (Birkenbihl *et al.*, 2012; Frerigmann and Gigolashvili, 2014; Frerigmann *et al.*, 2015). Consistently, three out of the four transcription factors appear to be substantially induced over the course of infection. However, glucobrassicin and camalexin originate from a common precursor molecule, indole-3-acetaldoxime (IAOx), which is assumed to represent an intermediate in a *Brassica*-specific metabolic shunt (Pollmann *et al.*, 2006; Lehmann *et al.*, 2017). Although some recent publications have suggested

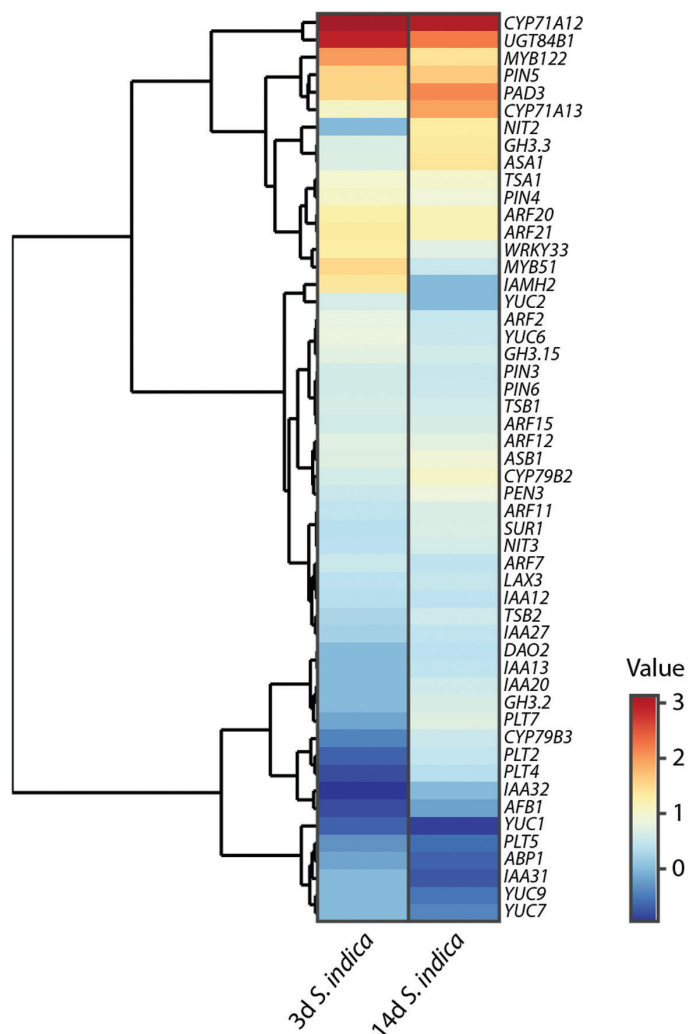


Fig. 3. Hierarchical clustering analysis of auxin-related genes differentially regulated during the first 2 weeks of co-cultivation with *S. indica*. The gene expression levels were extracted from publicly available data (GSE60736, Lahrmann *et al.*, 2015) deposited in the Gene Expression Omnibus (GEO) repository for high-throughput microarray and next-generation sequence functional genomic datasets (Barrett *et al.*, 2013). A *P*-value of 0.05 after adjustment for multiple testing and \log_2 ratio >0.75 were arbitrarily chosen to select 45 differentially expressed genes in Arabidopsis seedlings co-cultivated with *S. indica* relative to mock-treated control plants.

that IAOx also occurs outside the Brassicaceae (Irmisch *et al.*, 2013a, b; Luck *et al.*, 2016; Buezo *et al.*, 2019), a more general role for these compounds in the initial phase of the infection has to be doubted as long as a broader occurrence of IAOx in the plant kingdom is not unequivocally confirmed. Hence, metabolic engineering approaches on the basis of the IAOx shunt appear to offer only little prospect of improving agricultural productivity.

The induction of cytosolic calcium in plant–fungus interactions

The rapid detection of specific external stimuli, as well as a timely and adequate response to them, is often instrumental to guarantee plant survival. Second messenger molecules, such as

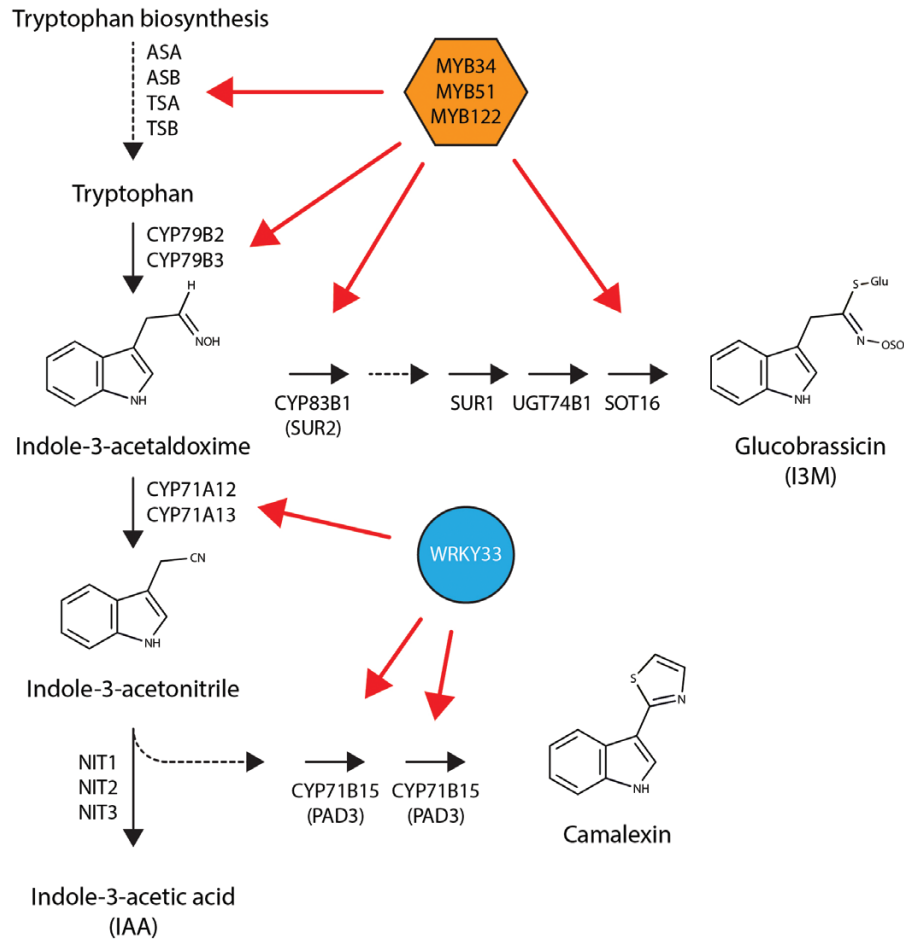


Fig. 4. Regulation of the glucobrassicin and camalexin biosynthetic pathways by MYB and WRKY transcription factors. Red arrows indicate experimentally proven gene regulatory interactions (Birkenbihl *et al.*, 2012; Frerigmann and Gigolashvili, 2014; Frerigmann *et al.*, 2015). Dashed lines indicate multi-step reactions; in some cases, not all enzymes are yet known. ASA, ANTHRANILATE SYNTHASE α ; ASB, ANTHRANILATE SYNTHASE β ; CYP, cytochrome P450 enzymes; I3M, indole-3-methyl glucosinolate; NIT1–3, NITRILASE 1–3; PAD3, PHYTOALEXIN DEFICIENT 3; SUR1–2, SUPERROOT 1–2; SOT16, SULFOTRANSFERASE 16; TSA, TRYPTOPHAN SYNTHASE α ; TSB, TRYPTOPHAN SYNTHASE β ; UGT74B1, UDP-GLYCOSYLTRANSFERASE 74B1

cyclic AMP or inositol triphosphate, are intracellular signaling molecules that are released by the cell in response to the perception of extracellular signals. They play important roles in the integration and transduction of signals, triggering physiological responses at the cellular level. Calcium (Ca^{2+}) is a highly conserved and very versatile intracellular second messenger linking several extracellular cues with appropriate cellular responses, including growth and defense (Dodd *et al.*, 2010). In addition, Ca^{2+} is involved in providing tolerance to biotic and abiotic stresses (Kudla *et al.*, 2018). Owing to the functional conservation of Ca^{2+} signaling in plants, it is not at all surprising that the recruitment of Ca^{2+} signaling by a colonizing fungus represents another well-characterized mechanism that plays a key role in the establishment of plant–fungus interactions.

The interaction of microbes with plant roots often involves a type of chemical communication that commences with the recognition of chemical mediators at the cell surface of the host plant and engages sophisticated downstream plant immunity mechanisms that act as surveillance systems to detect the invasion of microbes into the host cell. In the recognition process, microbe-associated molecular patterns (MAMPs) or

invasion patterns are perceived by specific receptors, which, in turn, activate pattern-triggered responses (van't Padje *et al.*, 2016; Zipfel and Oldroyd, 2017). The response cascades that are triggered can either be suppressed, for example, by biotrophs, or employed, in the case of necrotrophs, to continue symbiosis (Cook *et al.*, 2015).

Cell wall extracts (CWEs) from *S. indica* have been described to promote the elevation of cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{cyt}}$), thereby mimicking the presence of the fungus and promoting plant growth in the initial phase of the interaction (Vadassery *et al.*, 2009). Intriguingly, this observation suggests that growth promotion can be uncoupled from the colonization of the root system with *S. indica* and that growth promotion depends on Ca^{2+} . This is further supported by the fact that the CWE-mediated growth-promotion response can be blocked by the additional application of Ca^{2+} -specific chelators such as BAPTA or LaCl_3 . However, it should be noted that even repeated treatment of roots with CWEs has not been sufficient to entirely replace the interaction with the endosymbiont (Vadassery *et al.*, 2009). Hence, it may be concluded that further factors or processes, apart from the perception of the

active elicitor in the CWEs and the downstream Ca^{2+} signal, also play important roles in this context. The hydrolyzation of extracellular ATP (eATP) by specialized fungus-derived ecto-5'-nucleotidases and the resulting interference with the perception of eATP by the lectin receptor kinase DORN1 (Does not Respond to Nucleotides 1) could be such a missing process that apparently forms an integral part of the establishment of the symbiosis (Choi *et al.*, 2014a; Nizam *et al.*, 2019).

Mutant analyses revealed that CWE-mediated growth promotion in the *S. indica*–Arabidopsis interaction involves the activation of MITOGEN-ACTIVATED PROTEIN KINASE 6 (MAPK6), since the *mapk6* mutant shows no growth promotion upon treatment with CWEs. The Ca^{2+} influx-dependent activation of MAPK6 is induced by numerous microbial elicitors (Nühse *et al.*, 2000; Lecourieux *et al.*, 2002) and represents a common theme in plant resistance to biotic stresses (Menke *et al.*, 2004; Takahashi *et al.*, 2007). Furthermore, camalexin biosynthesis is reported to be controlled through the MAPK3/MAPK6 cascade, activating WRKY33 through phosphorylation (Ren *et al.*, 2008; Li *et al.*, 2012), which functionally links the increase of $[\text{Ca}^{2+}]_{\text{cyt}}$ to the observed plant defense response upon *S. indica* colonization (Figs 3, 4).

Recent work identified the molecular nature of the active elicitor in *S. indica* CWEs. During root colonization, cellotriose (CT) is released by the fungus to initiate the symbiotic interaction of the fungus with the root (Johnson *et al.*, 2018). Plant roots perceive and respond to very low concentrations of short-chain $\beta(1\rightarrow4)$ -linked D-glucose units (cellooligomers). Cellooligomers are generally released after structural changes to the cell wall caused by either environmental or plant integral signals. They are suggested to give an account of the state and integrity of the cell wall, which facilitates the activation of appropriate local and distal responses (Souza *et al.*, 2017; Oelmüller, 2018).

The perception of CT by an as yet unidentified receptor results in a dose-dependent rapid and transient increase in $[\text{Ca}^{2+}]_{\text{cyt}}$, which induces mild defense responses including the induction of reactive oxygen species, changes in membrane potential, and the expression of genes associated with growth regulation and root development (Johnson *et al.*, 2018). CT perception is independent of BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1), which is a well-known co-receptor contributing to the perception and integration of multiple MAMP-triggered responses, for instance, those toward bacterial flg22 or elf18 or fungal chitin, inducing $[\text{Ca}^{2+}]_{\text{cyt}}$ elevation and downstream immune responses (Lu *et al.*, 2010; Zhang *et al.*, 2010; Shi *et al.*, 2013; Li *et al.*, 2014; Kadota *et al.*, 2015). Moreover, mutants of the proposed Ca^{2+} channels GLU-LIKE RECEPTOR (GLR) 2.4, GLR2.5, GLR3.3, and the vacuolar TWO PORE CHANNEL 1 (TPC1) were not impaired in CT signaling. This strongly suggests that they are also not involved in the process, although they have previously been reported to contribute to wound signaling and wounding-induced systemic Ca^{2+} elevations (Peiter *et al.*, 2005; Li *et al.*, 2013; Manzoor *et al.*, 2013; Choi *et al.*, 2014b; Kiep *et al.*, 2015). However, the mechanisms that are involved in the rapid Ca^{2+} influx have yet to be identified.

In addition to the question of how the Ca^{2+} influx is brought about on the molecular level, the complexity of Ca^{2+} signaling, involving a large number of different Ca^{2+} -binding proteins to specifically decode incoming Ca^{2+} signals, makes it tremendously difficult to address the question of which molecular components contribute to the integration of the Ca^{2+} signals induced during *S. indica* infection. Complicating the situation even further, different Ca^{2+} signatures appear to trigger a variety of functions via the signature-specific activation of corresponding Ca^{2+} -sensor proteins (McAinsh and Pittman, 2009; Johnson *et al.*, 2011). All Ca^{2+} -sensor proteins, however, contain at least one so-called EF-hand motif (helix-loop-helix domain) in their primary sequence, which mediates the binding of Ca^{2+} to the sensor. The Arabidopsis proteome contains approximately 250 proteins with EF-hand motifs, and at least 100 have been classified as Ca^{2+} -sensor proteins (Day *et al.*, 2002; Hashimoto and Kudla, 2011). With respect to the nature of their response domain, which can be either a kinase or a transcription regulation domain, sensor proteins are further divided into sensor relay proteins or sensor responders. The latter class of sensors are particularly versatile, as they combine a sensor and response domain in a single protein and can thus directly transduce the Ca^{2+} signal to downstream target proteins by phosphorylation. CALCIUM-DEPENDENT PROTEIN KINASEs (CDPKs) represent an important family of these sensor responder proteins, and 34 members of this family have been identified in Arabidopsis (Cheng *et al.*, 2002). CDPKs have been demonstrated to be activated by Ca^{2+} signals during the course of interactions of plant roots with biotrophic microbes. They are speculated to control host entry and accommodation in the plant through the efficient suppression of corresponding plant defense responses (Freyermark *et al.*, 2007; Chen *et al.*, 2015a). Recent work has suggested the involvement of CDPKs in the *S. indica*–plant interaction as well, as *S. indica* CWE-mediated $[\text{Ca}^{2+}]_{\text{cyt}}$ elevation has been shown to promote tuberization in potato via a pathway that involves the potato CDPK1 sensor responder (Upadhyaya *et al.*, 2013). The work by Johnson *et al.* (2018) supports this hypothesis, because their microarray data provide strong evidence for the induction of a number of CDPK proteins upon CT treatment. A more detailed analysis of CDPKs involved in the establishment and maintenance of the fungus–plant symbiosis has yet to be carried out, although it might be very difficult to achieve this task owing to the large number of CDPK family members in plants and their presumably partially overlapping functions. Available microarray data also revealed the differential expression of a small number of sensor relay proteins—CALMODULIN-LIKE 37 (CML37) and CML38 as well as CBL-INTERACTING PROTEIN KINASE 13 (CIPK13) and CIPK20—but their role in fungus–plant interaction is even less well investigated and remains a matter of debate (Vahabi *et al.*, 2015; Johnson *et al.*, 2018).

***Serendipita indica* and the growth–defense tradeoff enigma**

The initial phase of root colonization by *S. indica* involves the mounting of defense responses by the host plant (Zuccaro

et al., 2011; Vahabi *et al.*, 2015; Johnson *et al.*, 2018). Such defense responses frequently come at the cost of a substantial reduction in growth and reproduction, which carries important implications for agriculture. However, despite the obvious importance of this tradeoff between growth and defense in shaping plant productivity in agricultural ecosystems, the molecular mechanisms that connect plant growth with plant defense responses are only poorly understood. From the classical point of view, plants employ physiological tradeoffs to allocate their limited metabolic resources between the generation of defense-related protective compounds on the one hand and associated morphological structures on the other hand. In other words, plants face a dilemma: to decide whether to grow or to defend themselves (Herms and Mattson, 1992; Züst *et al.*, 2015). Although most ecological studies of plant resistance to herbivores or pathogens still use the concept of growth–defense tradeoffs as their major paradigm (Denancé *et al.*, 2013; Huot *et al.*, 2014), more recent studies have questioned this simplistic view (Kliebenstein, 2016; Schuman and Baldwin, 2016). They point out that growth inhibition in response to, for example, herbivory is likely not the result of the simple channeling of photoassimilates from growth to defense but rather due to a conserved transcriptional network that serves the purpose of attenuating growth upon wounding (Campos *et al.*, 2016).

However, whichever point of view is chosen, it seems as if plant defense is always realized at the expense of growth, whether through the reallocation of metabolic resources or the activation of specific gene regulatory networks hardwired to confront biotic stress situations. When inspecting the beneficial effect of the co-cultivation of plants with *S. indica* it becomes obvious that there is a discrepancy between the observed effects and the classical growth–defense tradeoff concept. Although *S. indica* infection triggers defense responses, plant biomass production and productivity in terms of seed yield are increased (Oelmüller *et al.*, 2009; Achatz *et al.*, 2010). It therefore has to be concluded that, along with the basic initial plant defense response, further mechanisms are triggered by the fungus, allowing the host plant to grow despite all of the metabolic restrictions. In order to sustain growth without paying the price of increased stress susceptibility, the underlying mechanisms likely involve the improvement of plant nutrition, which is essential to mitigate the provoked metabolic deficit. The pronounced extension of the root system of infected plants (Fig. 2) suggests that the penetration and exploration of new areas of soil plays an important role in this context.

In their natural soil habitats, plants interact with a broad variety of microorganisms. In particular, microbes in the rhizosphere play important roles in the acquisition of soil nutrients, including the most important macronutrients, nitrogen (N) and phosphorus (P) (Hayat *et al.*, 2010; Hacquard *et al.*, 2015, 2017). Soil microbes can increase nutrient uptake by converting insoluble complexes, which represent unavailable forms of nutrients in the soil, to their ionic forms, which are more suitable for assimilation via the roots. In addition, the beneficial relationships of AMFs with their host plants have been extensively studied. The mycorrhizal symbiosis is known to facilitate improved access to soil nutrients,

particularly phosphate (Chiu and Paszkowski, 2019). As previously pointed out, *S. indica* and other Sebaciniales resemble AMFs in many aspects. Thus, it does not appear improbable that those mutualistic fungi may also impact nutrient uptake in their host plants, and indeed similar effects have been reported (Malla *et al.*, 2004; Shahollari *et al.*, 2005; Saddique *et al.*, 2018). In mycorrhizal communities, nutrient exchange involves a number of specific transporters for both the uptake of nutrients by the fungus and their subsequent exchange with the host (Wang *et al.*, 2017). For most other beneficial endophytic fungi, including *S. indica*, the molecular mechanisms involved in nutrient exchange are only poorly understood and need to be further investigated. Although it is generally assumed that an effective mechanism exists for nutrient absorption and translocation to the host plant by *S. indica*, in exchange for plant-derived carbon sources (Khalid *et al.*, 2019), the translocation of inorganic phosphate by *S. indica* is controversial. *S. indica* has been reported to contain a high-affinity phosphate transporter (PiPT) that is presumably involved in the transfer of inorganic phosphate to the host plant (Yadav *et al.*, 2010; Kumar *et al.*, 2011). However a more recent study claimed that *S. indica* interferes primarily with P_i distribution and metabolism, rather than directly promoting phosphate uptake from the soil (Bakshi *et al.*, 2017).

With respect to N, it has been suggested that the inoculation of Arabidopsis and *Nicotiana tabacum* with *S. indica* induces N uptake and translocation from the culture medium to the aerial parts of the plant. The reported induction of N uptake is seemingly linked to the stimulation of nitrate reductase activity, a key step in nitrate assimilation, and the transcriptional activation of *NITRATE REDUCTASE 2 (NIA2)* expression in Arabidopsis (Sherameti *et al.*, 2005). In summary, the available data support the idea that *S. indica* encroaches on the primary metabolism in host plant roots by delivering nutrients necessary for increased growth and development. The exact mechanisms by which *S. indica* achieves this, however, remain largely unknown.

Induced drought and salt resistance

In the global climate change scenario, plant growth and productivity are greatly affected by drought stress, and plants have to adapt to prevailing unfavorable conditions in order to survive (Shinozaki and Yamaguchi-Shinozaki, 2007). Drought provokes a number of interconnected physiological and biochemical responses, including stomatal closure, repression of plant growth and photosynthetic activity, and activation of respiration (Schroeder *et al.*, 2001; Flexas *et al.*, 2004; Roelfsema and Hedrich, 2005; Rennenberg *et al.*, 2006). Work conducted over recent years has identified a large number of drought-inducible genes, which can be divided into two major groups: (i) genes that encode proteins directly involved in conveying abiotic stress tolerance, and (ii) genes encoding regulatory proteins, which interfere with signal transduction or the expression of stress-responsive genes. ABA is known to play a central role in the conversion of abiotic stress signals into appropriate cellular responses, although some ABA-independent signaling

pathways have also been shown to contribute to the transcriptional control of abiotic stress–response genes.

S. indica was initially isolated from the roots of shrubs growing in the Thar desert in India. Hence, it is not surprising that this endosymbiont is also capable of attenuating the negative effects of drought stress effects in its host plant. Experiments with *Arabidopsis*, rice, and maize revealed that plants inoculated with *S. indica* performed considerably better under drought conditions (Sherameti *et al.*, 2008; Hosseini *et al.*, 2018; Saddique *et al.*, 2018; Zhang *et al.*, 2018). Microarray analyses performed by Zhang *et al.* (2018) indicated that a quite diverse set of stress–related genes (up to 2037 genes after 12 h of drought stress treatment) responded differentially in plants co–cultivated with *S. indica*. The detailed breakdown of the transcriptomic data revealed that *S. indica* promotes maize root growth under drought stress conditions largely through the stimulation of microtubular processes and the strengthening of the plant's redox capacity by adjusting its carbon–sulfur balance.

Apart from drought stress, soil salinity represents an increasingly serious environmental threat that affects plant growth and yield. Soil salinity disturbs nutrient absorption by the roots, leading to osmotic and ionic imbalances and oxidative damage (Flowers, 2004; Ruiz–Lozano *et al.*, 2012). Similar to drought stress, salt stress leads to stomatal closure because it reduces the water potential of leaves, which results in decreased photosynthetic activity and increased photodamage (Baker and Rosenqvist, 2004). Several studies have indicated that *S. indica* is able to increase plant tolerance to salinity, partly through the increased conservation of photosynthetic pigments to reduce photodamage (Waller *et al.*, 2005; Jogawat *et al.*, 2013; Sharma *et al.*, 2014; Ghorbani *et al.*, 2018). However, the precise mechanism by which *S. indica* improves plant growth under conditions of salt stress has yet to be elucidated. The most recent studies underpin the idea that *S. indica* colonization of roots improves potassium (K^+)/sodium (Na^+) homeostasis through the transcriptional regulation of the cyclic nucleotide–gated channel *CNGC15* and plant cation/proton antiporter (*NHX*) genes, including *SOS1*. In addition, the water uptake potential of host plant roots is increased by the transcriptional regulation of aquaporins, in particular PIPs (plasma membrane intrinsic proteins) and TIPs (tonoplast intrinsic proteins), which have been suggested to be involved in water use efficiency and the maintenance of water equilibrium in plants subjected to diverse environmental stresses (Sade *et al.*, 2010; Ghorbani *et al.*, 2019). Intriguingly, the ion homeostasis– and water status–related effects triggered in the host plant are accompanied by an active Na^+ detoxification of plant cells by *S. indica*. The reduction of Na^+ contents in plants colonized with *S. indica* under saline conditions has been attributed to the induction of two ENA ATPases, *SiENA1* and *SiENA5*, which are involved in K^+/Na^+ and Na^+ efflux, respectively (Lanza *et al.*, 2019). In consequence, this active Na^+ detoxification suggests the existence of a barrier effect evoked by *S. indica* that prevents the accumulation of cations in the plant root. Taken together, recent work sheds some light on the molecular bases and mechanisms of *S. indica*–mediated improved salt tolerance.

Nonetheless, there are still significant gaps in our current understanding of the processes involved, most importantly concerning the underlying gene regulatory networks.

Prospects and conclusions

Fungi of the order Sebaciales have been identified all around the world, including in extreme ecosystems such as deserts. The root endophyte *S. indica* is capable of associating with all plants tested so far, transferring growth benefits and increased stress tolerance to its host plants under a broad range of different climate, temperature, and growth conditions. As is evident from the data discussed in this review, the molecular mechanisms that confer the beneficial effects upon host plants are highly complex and multi–layered, which makes it unlikely that a single master regulator will be identified that could be used in biotechnological approaches to unleash the full repertoire of fungal effects exerted during symbiosis in transgenic plants. The increasing insight into the molecular bases of the plant–fungal symbiosis will most probably provide evidence for suitable target genes that drive isolated facets of the interaction, such as growth promotion or increased biotic and abiotic stress tolerance.

A particularly interesting starting point for the improvement of agricultural productivity using information gained from studies of plant–microbe interactions is provided by the identification of the cellooligomer CT, which is the active elicitor of $[Ca^{2+}]_{cyt}$ elevations in *S. indica* CWEs. Apart from the induction of diverse defense responses, cellular Ca^{2+} signatures have recently been reported to directly contribute to primary root growth and development (Leitão *et al.*, 2019). However, the application of CT does not provide the full effect of symbiosis, and additionally CT is far too expensive for application in the field to be cost–effective. Nevertheless, some more investigation in this direction, for example, by taking a chemical genetics approach to identify reagents that trigger similar physiological responses and can be applied to soils as plant biostimulants to improve plant productivity, would be valuable. Subsequent studies should include a comprehensive examination of the general applicability of identified compounds and the best way to formulate them. A formulation employing slowly degrading coated beads that persist over a long period of time, as is used for enhanced–efficiency fertilizers, could be an effective way to apply putative CT–like reagents (Timilsena *et al.*, 2015). By using such a formulation, the micro–dosage present in the symbiosis could be emulated. The biochemical nature of the compound(s) also has to be considered. CT itself is an energy–rich carbon source that can most likely be used by other saprophytic fungi present in the soil that are able to degrade the triose. In this respect, a slow–release formulation could also help to contain the growth of unwanted fungal pathogens through limiting its availability as a source of carbon. At best, the chemical genetics approach may provide evidence for chemical compounds with CT–like effects on crops that are not digestible by microbes and, therefore, do not represent an easily accessible carbon source. However, with respect to organic farming practices in particular, the application

of synthetic compounds to the field does not come without disadvantages.

In conclusion, the direct application of the fungus *S. indica* as a biostimulant is currently possibly the most suitable method to make use of the beneficial traits transferred by endophytic fungi to their host plants. *S. indica* offers the huge advantage that it will grow axenically and without a host plant. Moreover, it can be propagated at large scale and used as a biocontrol agent (Sun *et al.*, 2014), which underlines its high potential for biotechnological and agricultural applications.

Acknowledgements

The authors are grateful for financial assistance received from the collaborative IPSC research project realized 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. CGG was supported by the Severo Ochoa Program for Centers of Excellence in R&D from the Agencia Estatal de Investigación, Spain, grant SEV-2016-0672 (2017–2021) to the Centro de Biotecnología y Genómica de Plantas.

References

Abdelaziz ME, Abdelsattar M, Abdeldaym EA, Atia MAM, Mahmoud AWM, Saad MM, Hirt H. 2019. *Piriformospora indica* alters Na⁺/K⁺ homeostasis, antioxidant enzymes and *LeNHX1* expression of greenhouse tomato grown under salt stress. *Scientia Horticulturae* **256**, 108532.

Achatz B, Kogel KH, Franken P, Waller F. 2010. *Piriformospora indica* mycorrhization increases grain yield by accelerating early development of barley plants. *Plant Signaling & Behavior* **5**, 1685–1687.

Aglawe SB, Barbadikar KM, Mangrauthia SK, Madhav MS. 2018. New breeding technique “genome editing” for crop improvement: applications, potentials and challenges. *3 Biotech* **8**, 336.

Andrade-Linares DR, Grosch R, Franken P, Rexer KH, Kost G, Restrepo S, de Garcia MC, Maximova E. 2011. Colonization of roots of cultivated *Solanum lycopersicum* by dark septate and other ascomycetous endophytes. *Mycologia* **103**, 710–721.

Baker NR, Rosenqvist E. 2004. Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *Journal of Experimental Botany* **55**, 1607–1621.

Bakshi M, Sherameti I, Meichsner D, Thürich J, Varma A, Johri AK, Yeh KW, Oelmüller R. 2017. *Piriformospora indica* reprograms gene expression in Arabidopsis phosphate metabolism mutants but does not compensate for phosphate limitation. *Frontiers in Microbiology* **8**, 1262.

Barrett T, Wilhite SE, Ledoux P, *et al.* 2013. NCBI GEO: archive for functional genomics data sets—update. *Nucleic Acids Research* **41**, D991–D995.

Birkenbihl RP, Diezel C, Somssich IE. 2012. Arabidopsis WRKY33 is a key transcriptional regulator of hormonal and metabolic responses toward *Botrytis cinerea* infection. *Plant Physiology* **159**, 266–285.

Böttcher C, Dennis EG, Booker GW, Polyak SW, Boss PK, Davies C. 2012. A novel tool for studying auxin-metabolism: the inhibition of grapevine indole-3-acetic acid-amido synthetases by a reaction intermediate analogue. *PLoS One* **7**, e37632.

Böttcher C, Keyzers RA, Boss PK, Davies C. 2010. Sequestration of auxin by the indole-3-acetic acid-amido synthetase GH3-1 in grape berry (*Vitis vinifera* L.) and the proposed role of auxin conjugation during ripening. *Journal of Experimental Botany* **61**, 3615–3625.

Buezo J, Esteban R, Cornejo A, López-Gómez P, Marino D, Chamizo-Ampudia A, Gil MJ, Martínez-Merino V, Moran JF. 2019. IAOx induces the SUR phenotype and differential signalling from IAA under different types of nitrogen nutrition in *Medicago truncatula* roots. *Plant Science* **287**, 110176.

Campos ML, Yoshida Y, Major IT, *et al.* 2016. Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs. *Nature Communications* **7**, 12570.

Chen J, Gutjahr C, Bleckmann A, Dresselhaus T. 2015a. Calcium signaling during reproduction and biotrophic fungal interactions in plants. *Molecular Plant* **8**, 595–611.

Chen Q, Liu Y, Maere S, *et al.* 2015b. A coherent transcriptional feed-forward motif model for mediating auxin-sensitive *PIN3* expression during lateral root development. *Nature Communications* **6**, 8821.

Cheng SH, Willmann MR, Chen HC, Sheen J. 2002. Calcium signaling through protein kinases. The Arabidopsis calcium-dependent protein kinase gene family. *Plant Physiology* **129**, 469–485.

Chiu CH, Paszkowski U. 2019. Mechanisms and impact of symbiotic phosphate acquisition. *Cold Spring Harbor Perspectives in Biology* **11**, a034603.

Choi J, Tanaka K, Cao Y, Qi Y, Qiu J, Liang Y, Lee SY, Stacey G. 2014a. Identification of a plant receptor for extracellular ATP. *Science* **343**, 290–294.

Choi WG, Toyota M, Kim SH, Hilleary R, Gilroy S. 2014b. Salt stress-induced Ca²⁺ waves are associated with rapid, long-distance root-to-shoot signaling in plants. *Proceedings of the National Academy of Sciences, USA* **111**, 6497–6502.

Cook DE, Mesarich CH, Thomma BP. 2015. Understanding plant immunity as a surveillance system to detect invasion. *Annual Review of Phytopathology* **53**, 541–563.

Davies PJ. 2010. *Plant hormones. biosynthesis, signal transduction, action!* Dordrecht: Springer Netherlands.

Day IS, Reddy VS, Shad Ali G, Reddy AS. 2002. Analysis of EF-hand-containing proteins in *Arabidopsis*. *Genome Biology* **3**, RESEARCH0056.

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

Denancé N, Sánchez-Vallet A, Goffner D, Molina A. 2013. Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. *Frontiers in Plant Science* **4**, 155.

Deshmukh S, Hückelhoven R, Schäfer P, Imani J, Sharma M, Weiss M, Waller F, Kogel KH. 2006. The root endophytic fungus *Piriformospora indica* requires host cell death for proliferation during mutualistic symbiosis with barley. *Proceedings of the National Academy of Sciences, USA* **103**, 18450–18457.

Dodd AN, Kudla J, Sanders D. 2010. The language of calcium signaling. *Annual Review of Plant Biology* **61**, 593–620.

FAO, IFAD, WFP. 2015. *The state of food insecurity in the world 2015*. Rome: Food and Agriculture Organization of the United Nations.

Fedoroff NV. 2015. Food in a future of 10 billion. *Agriculture & Food Security* **4**, 11.

Flexas J, Bota J, Loreto F, Cornic G, Sharkey TD. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C₃ plants. *Plant Biology* **6**, 269–279.

Flowers TJ. 2004. Improving crop salt tolerance. *Journal of Experimental Botany* **55**, 307–319.

Frigemann H, Gigolashvili T. 2014. MYB34, MYB51, and MYB122 distinctly regulate indolic glucosinolate biosynthesis in *Arabidopsis thaliana*. *Molecular Plant* **7**, 814–828.

Frigemann H, Glawischnig E, Gigolashvili T. 2015. The role of MYB34, MYB51 and MYB122 in the regulation of camalexin biosynthesis in *Arabidopsis thaliana*. *Frontiers in Plant Science* **6**, 654.

Freyermark G, Diehl T, Miklis M, Romeis T, Panstruga R. 2007. Antagonistic control of powdery mildew host cell entry by barley calcium-dependent protein kinases (CDPKs). *Molecular Plant-Microbe Interactions* **20**, 1213–1221.

Friml J, Benková E, Blilou I, *et al.* 2002. AtPIN4 mediates sink-driven auxin gradients and root patterning in *Arabidopsis*. *Cell* **108**, 661–673.

Fu SF, Wei JY, Chen HW, Liu YY, Lu HY, Chou JY. 2015. Indole-3-acetic acid: a widespread physiological code in interactions of fungi with other organisms. *Plant Signaling & Behavior* **10**, e1048052.

- Gartland KMA, Gartland JS.** 2018. Opportunities in biotechnology. *Journal of Biotechnology* **282**, 38–45.
- Ghorbani A, Omran VOG, Razavi SM, Pirdashti H, Ranjbar M.** 2019. *Piriformospora indica* confers salinity tolerance on tomato (*Lycopersicon esculentum* Mill.) through amelioration of nutrient accumulation, K⁺/Na⁺ homeostasis and water status. *Plant Cell Reports* **38**, 1151–1163.
- Ghorbani A, Razavi SM, Ghasemi Omran VO, Pirdashti H.** 2018. *Piriformospora indica* inoculation alleviates the adverse effect of NaCl stress on growth, gas exchange and chlorophyll fluorescence in tomato (*Solanum lycopersicum* L.). *Plant Biology* **20**, 729–736.
- Gill SS, Gill R, Trivedi DK, et al.** 2016. *Piriformospora indica*: potential and significance in plant stress tolerance. *Frontiers in Microbiology* **7**, 332.
- Hacquard S, Garrido-Oter R, González A, et al.** 2015. Microbiota and host nutrition across plant and animal kingdoms. *Cell Host & Microbe* **17**, 603–616.
- Hacquard S, Spaepen S, Garrido-Oter R, Schulze-Lefert P.** 2017. Interplay between innate immunity and the plant microbiota. *Annual Review of Phytopathology* **55**, 565–589.
- Harman GE.** 2011. Multifunctional fungal plant symbionts: new tools to enhance plant growth and productivity. *New Phytologist* **189**, 647–649.
- Hashimoto K, Kudla J.** 2011. Calcium decoding mechanisms in plants. *Biochimie* **93**, 2054–2059.
- Hayat R, Ali S, Amara U, Khalid R, Ahmed I.** 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology* **60**, 579–598.
- Hermes DA, Mattson WJ.** 1992. The dilemma of plants: to grow or defend. *Quarterly Review of Biology* **67**, 283–335.
- Hertig M, Taliaferro WH, Schwartz B.** 1937. The terms symbiosis, symbiont and symbiote. *Journal of Parasitology* **23**, 326–329.
- Hilbert M, Voll LM, Ding Y, Hofmann J, Sharma M, Zuccaro A.** 2012. Indole derivative production by the root endophyte *Piriformospora indica* is not required for growth promotion but for biotrophic colonization of barley roots. *New Phytologist* **196**, 520–534.
- Hosseini F, Mosaddeghi MR, Dexter AR, Sepehri M.** 2018. Maize water status and physiological traits as affected by root endophytic fungus *Piriformospora indica* under combined drought and mechanical stresses. *Planta* **247**, 1229–1245.
- Hua MD, Senthil Kumar R, Shyur LF, Cheng YB, Tian Z, Oelmüller R, Yeh KW.** 2017. Metabolomic compounds identified in *Piriformospora indica*-colonized Chinese cabbage roots delineate symbiotic functions of the interaction. *Scientific Reports* **7**, 9291.
- Huot B, Yao J, Montgomery BL, He SY.** 2014. Growth–defense tradeoffs in plants: a balancing act to optimize fitness. *Molecular Plant* **7**, 1267–1287.
- Irmisch S, McCormick AC, Boeckler GA, et al.** 2013a. Two herbivore-induced cytochrome P450 enzymes CYP79D6 and CYP79D7 catalyze the formation of volatile aldoximes involved in poplar defense. *The Plant Cell* **25**, 4737–4754.
- Irmisch S, Unsicker SB, Gershenzon J, Köllner TG.** 2013b. Identification and characterization of CYP79D6v4, a cytochrome P450 enzyme producing aldoximes in black poplar (*Populus nigra*). *Plant Signaling & Behavior* **8**, e27640.
- ISAAA.** 2017. *Pocket K No. 4: GM crops and the environment*. Los Baños, Laguna, International Service for the Acquisition of Agri-biotech Applications.
- Jackson RG, Kowalczyk M, Li Y, Higgins G, Ross J, Sandberg G, Bowles DJ.** 2002. Over-expression of an *Arabidopsis* gene encoding a glucosyltransferase of indole-3-acetic acid: phenotypic characterisation of transgenic lines. *The Plant Journal* **32**, 573–583.
- Jackson RG, Lim EK, Li Y, Kowalczyk M, Sandberg G, Hoggett J, Ashford DA, Bowles DJ.** 2001. Identification and biochemical characterization of an *Arabidopsis* indole-3-acetic acid glucosyltransferase. *Journal of Biological Chemistry* **276**, 4350–4356.
- Jacobs S, Zechmann B, Molitor A, Trujillo M, Petutschnig E, Lipka V, Likpa V, Kogel KH, Schäfer P.** 2011. Broad-spectrum suppression of innate immunity is required for colonization of *Arabidopsis* roots by the fungus *Piriformospora indica*. *Plant Physiology* **156**, 726–740.
- Jogawat A, Saha S, Bakshi M, Dayaman V, Kumar M, Dua M, Varma A, Oelmüller R, Tuteja N, Johri AK.** 2013. *Piriformospora indica* rescues growth diminution of rice seedlings during high salt stress. *Plant Signaling & Behavior* **8**, e26891.
- Johnson JM, Nongbri PL, Sherameti I, Oelmüller R.** 2011. Calcium signaling and cytosolic calcium measurements in plants. *Endocytobiosis and Cell Research* **21**, 64–76.
- Johnson JM, Thürich J, Petutschnig EK, et al.** 2018. A poly(A) ribonuclease controls the cellobiose-based interaction between *Piriformospora indica* and its host arabidopsis. *Plant Physiology* **176**, 2496–2514.
- Jumpponen A, Trappe JM.** 1998. Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytologist* **140**, 295–310.
- Kadota Y, Shirasu K, Zipfel C.** 2015. Regulation of the NADPH oxidase RBOHD during plant immunity. *Plant & Cell Physiology* **56**, 1472–1480.
- Khalid M, Rahman SU, Huang D.** 2019. Molecular mechanism underlying *Piriformospora indica*-mediated plant improvement/protection for sustainable agriculture. *Acta Biochimica et Biophysica Sinica* **51**, 229–242.
- Kiep V, Vadassery J, Lattke J, Maaß JP, Boland W, Peiter E, Mithöfer A.** 2015. Systemic cytosolic Ca²⁺ elevation is activated upon wounding and herbivory in *Arabidopsis*. *New Phytologist* **207**, 996–1004.
- Kliebenstein DJ.** 2016. False idolatry of the mythical growth versus immunity tradeoff in molecular systems plant pathology. *Physiological and Molecular Plant Pathology* **95**, 55–59.
- Kudla J, Becker D, Grill E, Hedrich R, Hippler M, Kummer U, Parniske M, Romeis T, Schumacher K.** 2018. Advances and current challenges in calcium signaling. *New Phytologist* **218**, 414–431.
- Kumar M, Yadav V, Kumar H, Sharma R, Singh A, Tuteja N, Johri AK.** 2011. *Piriformospora indica* enhances plant growth by transferring phosphate. *Plant Signaling & Behavior* **6**, 723–725.
- Lahrman U, Strehmel N, Langen G, Frerigmann H, Leson L, Ding Y, Scheel D, Herklotz S, Hilbert M, Zuccaro A.** 2015. Mutualistic root endophytism is not associated with the reduction of saprotrophic traits and requires a noncompromised plant innate immunity. *New Phytologist* **207**, 841–857.
- Lanza M, Haro R, Conchillo LB, Benito B.** 2019. The endophyte *Serendipita indica* reduces the sodium content of *Arabidopsis* plants exposed to salt stress: fungal ENA ATPases are expressed and regulated at high pH and during plant co-cultivation in salinity. *Environmental Microbiology* **21**, 3364–3378.
- Laskowski M, Ten Tusscher KH.** 2017. Periodic lateral root priming: what makes it tick? *The Plant Cell* **29**, 432–444.
- Lecourieux D, Mazars C, Pauly N, Ranjeva R, Pugin A.** 2002. Analysis and effects of cytosolic free calcium increases in response to elicitors in *Nicotiana plumbaginifolia* cells. *The Plant Cell* **14**, 2627–2641.
- Lee YC, Johnson JM, Chien CT, Sun C, Cai D, Lou B, Oelmüller R, Yeh KW.** 2011. Growth promotion of Chinese cabbage and *Arabidopsis* by *Piriformospora indica* is not stimulated by mycelium-synthesized auxin. *Molecular Plant-Microbe Interactions* **24**, 421–431.
- Lehmann T, Janowitz T, Sánchez-Parra B, Alonso MP, Trompeter I, Piotrowski M, Pollmann S.** 2017. *Arabidopsis* NITRILASE 1 contributes to the regulation of root growth and development through modulation of auxin biosynthesis in seedlings. *Frontiers in Plant Science* **8**, 36.
- Leitão N, Dangeville P, Carter R, Charpentier M.** 2019. Nuclear calcium signatures are associated with root development. *Nature Communications* **10**, 4865.
- Li F, Wang J, Ma C, Zhao Y, Wang Y, Hasi A, Qi Z.** 2013. Glutamate receptor-like channel3.3 is involved in mediating glutathione-triggered cytosolic calcium transients, transcriptional changes, and innate immunity responses in *Arabidopsis*. *Plant Physiology* **162**, 1497–1509.
- Li G, Meng X, Wang R, Mao G, Han L, Liu Y, Zhang S.** 2012. Dual-level regulation of ACC synthase activity by MPK3/MPK6 cascade and its downstream WRKY transcription factor during ethylene induction in *Arabidopsis*. *PLOS Genetics* **8**, e1002767.
- Li L, Li M, Yu L, et al.** 2014. The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RbohD to control plant immunity. *Cell Host & Microbe* **15**, 329–338.
- Liu H, Senthilkumar R, Ma G, Zou Q, Zhu K, Shen X, Tian D, Hua MS, Oelmüller R, Yeh KW.** 2019. *Piriformospora indica*-induced phytohormone changes and root colonization strategies are highly host-specific. *Plant Signaling & Behavior* **14**, 1632688.
- Lu D, Wu S, Gao X, Zhang Y, Shan L, He P.** 2010. A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. *Proceedings of the National Academy of Sciences, USA* **107**, 496–501.

- Luck K, Jirschitzka J, Irmisch S, Huber M, Gershenzon J, Köllner TG.** 2016. CYP79D enzymes contribute to jasmonic acid-induced formation of aldoximes and other nitrogenous volatiles in two *Erythroxylum* species. *BMC Plant Biology* **16**, 215.
- Malla R, Prasad R, Kumari R, Giang PH, Pokharel U, Oelmüller R, Varma A.** 2004. Phosphorus solubilizing symbiotic fungus: *Piriformospora indica*. *Endocytobiosis Cell Research* **15**, 579–600.
- Manzoor H, Kelloniemi J, Chiltz A, Wendehenne D, Pugin A, Poinssot B, Garcia-Brugger A.** 2013. Involvement of the glutamate receptor AtGLR3.3 in plant defense signaling and resistance to *Hyaloperonospora arabidopsidis*. *The Plant Journal* **76**, 466–480.
- McAinsh MR, Pittman JK.** 2009. Shaping the calcium signature. *New Phytologist* **181**, 275–294.
- Meents AK, Furch ACU, Almeida-Trapp M, et al.** 2019. Beneficial and pathogenic *Arabidopsis* root-interacting fungi differently affect auxin levels and responsive genes during early infection. *Frontiers in Microbiology* **10**, 380.
- Menke FL, van Pelt JA, Pieterse CM, Klessig DF.** 2004. Silencing of the mitogen-activated protein kinase MPK6 compromises disease resistance in *Arabidopsis*. *The Plant Cell* **16**, 897–907.
- Molina-Montenegro MA, Oses R, Torres-Diaz C, Atala C, Zurita-Silva A, Ruiz-Lara S.** 2016. Root-endophytes improve the ecophysiological performance and production of an agricultural species under drought condition. *AoB Plants* **8**, plw062.
- Mravec J, Skúpa P, Bailly A, et al.** 2009. Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. *Nature* **459**, 1136–1140.
- Murray DR.** 1997. *Carbon dioxide and plant responses*. Taunton, Research Studies Press.
- Nizam S, Qiang X, Wawra S, Nostadt R, Getzke F, Schwanke F, Dreyer I, Langen G, Zuccaro A.** 2019. *Serendipita indica* E5'NT modulates extracellular nucleotide levels in the plant apoplast and affects fungal colonization. *EMBO Reports* **20**, e47430.
- Nongbri PL, Johnson JM, Sherameti I, Glawischign E, Halkier BA, Oelmüller R.** 2012. Indole-3-acetaldoxime-derived compounds restrict root colonization in the beneficial interaction between *Arabidopsis* roots and the endophyte *Piriformospora indica*. *Molecular Plant-Microbe Interactions* **25**, 1186–1197.
- Nühse TS, Peck SC, Hirt H, Boller T.** 2000. Microbial elicitors induce activation and dual phosphorylation of the *Arabidopsis thaliana* MAPK 6. *Journal of Biological Chemistry* **275**, 7521–7526.
- Oelmüller R.** 2018. Sensing environmental and developmental signals via celooligomers. *Journal of Plant Physiology* **229**, 1–6.
- Oelmüller R, Sherameti I, Tripathi S, Varma A.** 2009. *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. *Symbiosis* **49**, 1–17.
- Overvoorde P, Fukaki H, Beekman T.** 2010. Auxin control of root development. *Cold Spring Harbor Perspectives in Biology* **2**, a001537.
- Peiter E, Maathuis FJ, Mills LN, Knight H, Pelloux J, Hetherington AM, Sanders D.** 2005. The vacuolar Ca²⁺-activated channel TPC1 regulates germination and stomatal movement. *Nature* **434**, 404–408.
- Peškan-Berghöfer T, Shahollari B, Gieng PH, Hehl S, Markert C, Blanke V, Kost G, Varma A, Oelmüller R.** 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**, 465–477.
- Petit JR, Jouzel J, Raynaud D, et al.** 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* **399**, 429–436.
- Pollmann S, Müller A, Weiler EW.** 2006. Many roads lead to “auxin”: of nitrilases, synthases, and amidases. *Plant Biology* **8**, 326–333.
- Qiang X, Weiss M, Kogel KH, Schäfer P.** 2012a. *Piriformospora indica*—a mutualistic basidiomycete with an exceptionally large plant host range. *Molecular Plant Pathology* **13**, 508–518.
- Qiang X, Zechmann B, Reitz MU, Kogel KH, Schäfer P.** 2012b. The mutualistic fungus *Piriformospora indica* colonizes *Arabidopsis* roots by inducing an endoplasmic reticulum stress-triggered caspase-dependent cell death. *The Plant Cell* **24**, 794–809.
- Ramos P, Rivas N, Pollmann S, Casati P, Molina-Montenegro MA.** 2018. Hormonal and physiological changes driven by fungal endophytes increase Antarctic plant performance under UV-B radiation. *Fungal Ecology* **34**, 76–82.
- Ren D, Liu Y, Yang KY, Han L, Mao G, Glazebrook J, Zhang S.** 2008. A fungal-responsive MAPK cascade regulates phytoalexin biosynthesis in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* **105**, 5638–5643.
- Rennenberg H, Loreto F, Polle A, Brilli F, Fares S, Beniwal RS, Gessler A.** 2006. Physiological responses of forest trees to heat and drought. *Plant Biology* **8**, 556–571.
- Rodríguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim YO, Redman RS.** 2008. Stress tolerance in plants via habitat-adapted symbiosis. *The ISME Journal* **2**, 404–416.
- Rodríguez RJ, Redman RS, Henson JM.** 2004. The role of fungal symbioses in the adaptation of plants to high stress environments. *Mitigation and Adaptation Strategies for Global Change* **9**, 261–272.
- Roelfsema MR, Hedrich R.** 2005. In the light of stomatal opening: new insights into ‘the Watergate’. *New Phytologist* **167**, 665–691.
- Ruiz-Lozano JM, Porcel R, Azcón C, Aroca R.** 2012. Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. *Journal of Experimental Botany* **63**, 4033–4044.
- Saddique MAB, Ali Z, Khan AS, Rana IA, Shamsi IH.** 2018. Inoculation with the endophyte *Piriformospora indica* significantly affects mechanisms involved in osmotic stress in rice. *Rice* **11**, 34.
- Sade N, Gebretsadik M, Seligmann R, Schwartz A, Wallach R, Moshelion M.** 2010. The role of tobacco Aquaporin1 in improving water use efficiency, hydraulic conductivity, and yield production under salt stress. *Plant Physiology* **152**, 245–254.
- Schäfer P, Piffi S, Voll LM, et al.** 2009. Manipulation of plant innate immunity and gibberellin as factor of compatibility in the mutualistic association of barley roots with *Piriformospora indica*. *The Plant Journal* **59**, 461–474.
- Schroeder JI, Kwak JM, Allen GJ.** 2001. Guard cell abscisic acid signalling and engineering drought hardiness in plants. *Nature* **410**, 327–330.
- Schuman MC, Baldwin IT.** 2016. The layers of plant responses to insect herbivores. *Annual Review of Entomology* **61**, 373–394.
- Shahollari B, Varma A, Oelmüller R.** 2005. Expression of a receptor kinase in *Arabidopsis* roots is stimulated by the basidiomycete *Piriformospora indica* and the protein accumulates in Triton X-100 insoluble plasma membrane microdomains. *Journal of Plant Physiology* **162**, 945–958.
- Sharma P, Kharkwal AC, Abdin MZ, Varma A.** 2014. *Piriformospora indica* improves micropropagation, growth and phytochemical content of *Aloe vera* L. plants. *Symbiosis* **64**, 11–23.
- Sherameti I, Shahollari B, Venus Y, Altschmied L, Varma A, Oelmüller R.** 2005. The endophytic fungus *Piriformospora indica* stimulates the expression of nitrate reductase and the starch-degrading enzyme glucan-water dikinase in tobacco and *Arabidopsis* roots through a homeodomain transcription factor that binds to a conserved motif in their promoters. *Journal of Biological Chemistry* **280**, 26241–26247.
- Sherameti I, Tripathi S, Varma A, Oelmüller R.** 2008. The root-colonizing endophyte *Piriformospora indica* confers drought tolerance in *Arabidopsis* by stimulating the expression of drought stress-related genes in leaves. *Molecular Plant-Microbe Interactions* **21**, 799–807.
- Shi H, Shen Q, Qi Y, Yan H, Nie H, Chen Y, Zhao T, Katagiri F, Tang D.** 2013. BR-SIGNALING KINASE1 physically associates with FLAGELLIN SENSING2 and regulates plant innate immunity in *Arabidopsis*. *The Plant Cell* **25**, 1143–1157.
- Shinozaki K, Yamaguchi-Shinozaki K.** 2007. Gene networks involved in drought stress response and tolerance. *Journal of Experimental Botany* **58**, 221–227.
- Souza CA, Li S, Lin AZ, Boutrot F, Grossmann G, Zipfel C, Somerville SC.** 2017. Cellulose-derived oligomers act as damage-associated molecular patterns and trigger defense-like responses. *Plant Physiology* **173**, 2383–2398.
- Spaepen S, Vanderleyden J, Remans R.** 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiology Reviews* **31**, 425–448.
- Staswick PE, Serban B, Rowe M, Tiryaki I, Maldonado MT, Maldonado MC, Souza W.** 2005. Characterization of an *Arabidopsis* enzyme family that conjugates amino acids to indole-3-acetic acid. *The Plant Cell* **17**, 616–627.

- Strehmel N, Monchgesang S, Herklotz S, Kruger S, Ziegler J, Scheel D.** 2016. *Piriformospora indica* stimulates root metabolism of *Arabidopsis thaliana*. *International Journal of Molecular Sciences* **17**, 1091.
- Su ZZ, Wang T, Shrivastava N, Chen YY, Liu X, Sun C, Yin Y, Gao QK, Lou BG.** 2017. *Piriformospora indica* promotes growth, seed yield and quality of *Brassica napus* L. *Microbiological Research* **199**, 29–39.
- Sun C, Shao Y, Vahabi K, et al.** 2014. The beneficial fungus *Piriformospora indica* protects *Arabidopsis* from *Verticillium dahliae* infection by downregulation plant defense responses. *BMC Plant Biology* **14**, 268.
- Takahashi F, Yoshida R, Ichimura K, Mizoguchi T, Seo S, Yonezawa M, Maruyama K, Yamaguchi-Shinozaki K, Shinozaki K.** 2007. The mitogen-activated protein kinase cascade MKK3–MPK6 is an important part of the jasmonate signal transduction pathway in *Arabidopsis*. *The Plant Cell* **19**, 805–818.
- Thürich J, Meichsner D, Furch ACU, Pfalz J, Krüger T, Kniemeyer O, Brakhage A, Oelmüller R.** 2018. *Arabidopsis thaliana* responds to colonisation of *Piriformospora indica* by secretion of symbiosis-specific proteins. *PLoS One* **13**, e0209658.
- Timilsena YP, Adhikari R, Casey P, Muster T, Gill H, Adhikari B.** 2015. Enhanced efficiency fertilisers: a review of formulation and nutrient release patterns. *Journal of the Science of Food and Agriculture* **95**, 1131–1142.
- Upadhyaya CP, Gururani MA, Prasad R, Verma A.** 2013. A cell wall extract from *Piriformospora indica* promotes tuberization in potato (*Solanum tuberosum* L.) via enhanced expression of Ca²⁺ signaling pathway and lipoxygenase gene. *Applied Biochemistry and Biotechnology* **170**, 743–755.
- Vadassery J, Ranf S, Drzewiecki C, Mithöfer A, Mazars C, Scheel D, Lee J, Oelmüller R.** 2009. A cell wall extract from the endophytic fungus *Piriformospora indica* promotes growth of *Arabidopsis* seedlings and induces intracellular calcium elevation in roots. *The Plant Journal* **59**, 193–206.
- Vadassery J, Ritter C, Venus Y, Camehl I, Varma A, Shahollari B, Novák O, Strnad M, Ludwig-Müller J, Oelmüller R.** 2008. The role of auxins and cytokinins in the mutualistic interaction between *Arabidopsis* and *Piriformospora indica*. *Molecular Plant-Microbe Interactions* **21**, 1371–1383.
- Vahabi K, Sherameti I, Bakshi M, Mrozinska A, Ludwig A, Reichelt M, Oelmüller R.** 2015. The interaction of *Arabidopsis* with *Piriformospora indica* shifts from initial transient stress induced by fungus-released chemical mediators to a mutualistic interaction after physical contact of the two symbionts. *BMC Plant Biology* **15**, 58.
- van't Padje A, Whiteside MD, Kiers ET.** 2016. Signals and cues in the evolution of plant-microbe communication. *Current Opinion in Plant Biology* **32**, 47–52.
- Varma A, Savita Verma, Sudha, Sahay N, Butehorn B, Franken P.** 1999. *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. *Applied and Environmental Microbiology* **65**, 2741–2744.
- Verma S, Varma A, Rexer K-H, Hassel A, Kost G, Sarbhoy A, Bisen P, Butehorn B, Franken P.** 1998. *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. *Mycologia* **90**, 896–903.
- Waller F, Achatz B, Baltruschat H, et al.** 2005. The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proceedings of the National Academy of Sciences, USA* **102**, 13386–13391.
- Wang W, Shi J, Xie Q, Jiang Y, Yu N, Wang E.** 2017. Nutrient exchange and regulation in arbuscular mycorrhizal symbiosis. *Molecular Plant* **10**, 1147–1158.
- Weiss M, Selosse MA, Rexer KH, Urban A, Oberwinkler F.** 2004. *Sebacinales*: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. *Mycological Research* **108**, 1003–1010.
- Weiß M, Sýkorová Z, Garnica S, Riess K, Martos F, Krause C, Oberwinkler F, Bauer R, Redecker D.** 2011. *Sebacinales* everywhere: previously overlooked ubiquitous fungal endophytes. *PLoS One* **6**, e16793.
- Weiß M, Waller F, Zuccaro A, Selosse MA.** 2016. *Sebacinales* - one thousand and one interactions with land plants. *New Phytologist* **211**, 20–40.
- Yadav V, Kumar M, Deep DK, Kumar H, Sharma R, Tripathi T, Tuteja N, Saxena AK, Johri AK.** 2010. A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant. *Journal of Biological Chemistry* **285**, 26532–26544.
- Zhang J, Li W, Xiang T, et al.** 2010. Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a *Pseudomonas syringae* effector. *Cell Host & Microbe* **7**, 290–301.
- Zhang W, Wang J, Xu L, Wang A, Huang L, Du H, Qiu L, Oelmüller R.** 2018. Drought stress responses in maize are diminished by *Piriformospora indica*. *Plant Signaling & Behavior* **13**, e1414121.
- Zipfel C, Oldroyd GE.** 2017. Plant signalling in symbiosis and immunity. *Nature* **543**, 328–336.
- Zuccaro A, Basiewicz M, Zurawska M, Biedenkopf D, Kogel KH.** 2009. Karyotype analysis, genome organization, and stable genetic transformation of the root colonizing fungus *Piriformospora indica*. *Fungal Genetics and Biology* **46**, 543–550.
- Zuccaro A, Lahrmann U, Güldener U, et al.** 2011. Endophytic life strategies decoded by genome and transcriptome analyses of the mutualistic root symbiont *Piriformospora indica*. *PLOS Pathogens* **7**, e1002290.
- Züst T, Rasmann S, Agrawal AA.** 2015. Growth–defense tradeoffs for two major anti-herbivore traits of the common milkweed *Asclepias syriaca*. *Oikos* **124**, 1404–1415.