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Review Article

Piriformospora indica: a root endophytic fungus and its roles in plants

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Abstract

Piriformospora indica is a discovered endophytic fungus colonizing in roots of plants in 1998. The fungus can form the mycelium, mycelial roll, and pear-shaped spores in intercellular and intracellular regions of roots. The fungus colonizes various host plants and also realizes the pure culture in vitro without roots of host plants. *P. indica* shows many positive effects on host plants, including the promotion of plant growth, the enhancement of nutrient acquisition and stress tolerance, the improvement of disease resistance, and the promoted accumulation of bioactive substances. The commercial production of the fungal spores is established in bioreactor with nanostructured materials "zinc oxide" as nano embedded fungus, which provides provides changes into confers. The review simply summarized the biological characteristics of *P. indica*, physiological roles in plants, and potential utilization as a biofertilizer.

Keywords: biofertilizer; endophytic fungus; *Piriformospora indica*; symbiotic interaction

Introduction

In 1998, *Piriformospora indica* Varma, Rexer, Kost & Franken sp. nov., an endophytic fungus of the Sebacinaceae family, was found in the rhizosphere of woody shrubs *Prosopis juliflora* and *Zizyphus nummularia* in the Tar Desert in Northwest India (Verma *et al*., 1998). The endophytic fungus is characterized by the formation of chlamydospore only with pear-shaped, originated from thin-walled vesicles at the tips of the hyphae (Verma *et al*., 1998). *P. indica* can grow on artificial media without plant roots and complete its life cycle. It is documented that about 150 plant species could establish symbiotic association with *P. indica*, and the fungus also colonizes non-mycorrhizal plants, including cruciferous crops plants, *Arabidopsis thaliana*, and so on (Liang *et al*., 2009).

In general, the young mycelium of *P. indica* is white and almost transparent with the diameter of 0.7-3.5 μm (Verma *et al*., 1998). The mycelium does not form aerial mycelium, whereas mycelium fusion can be quickly formed (Sherameti *et al*., 2008). The outer wall of the mycelium is separated and protruded, showing a multi-layer structure. The average thickness of the mycelium wall is about 0.3 μm. The chlamydospore produced by mature hyphae is the key feature of *P. indica* identification, whose shape is pear-shaped. The chlamydospore cytoplasm is filled with granular substances with 8-25 nuclei (Verma *et al*., 1998). The chlamydospores exist in a single or in clusters, whose length and width are about 16-45 μm and 10 μm, respectively. The spore wall

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thickness is about 0.7 μm, and after maturation the spore wall thickness can reach 1.5 μm. The chlamydospore has two layers of membranes, whilst the outer wall is pale yellow and the texture is relatively smooth.

The whole genome-sequence of *P. indica* was determined (Alga *et al*., 2011), having 1884 scaffolds (size 1 Kb; N50; 51.83Kb) with a size of 24.98 Mb. There are 2359 overlapping groups, each of which contains a reading coverage area with an average size of 22. The average number of exons per gene in *P. indica* is about 5.16, with a density of 471, a GC content of 50.68% and a repetition rate of 4.68%. The content of nucleus of DNA in the *P. indica* is about 15.3-21.3 Mb. The genome contains small secretory proteins encoded by different effector genes. The conserved region at different C-terminal heights has a gene family coding "DELD", which is related to the symbiotic nature of the bacteria. Such genomic information can provide the support for understanding the biological characteristics of the fungi and subsequent genetic studies.

Many studies had shown that *P. indica* could stimulate growth of various crop plants, accelerate nutrient acquisition, and enhance tolerance of plants to stress, thereby, having a very promising prospect for sustainable agriculture and environment (Waller *et al*., 2005; Liang *et al*., 2009). This review focuses on clarifying culture methods, interactions with plants, and potential application in agriculture for *P. indica*.

Culture of Piriformospora indica

The culture of *P. india* is usually carried out on potato glucose solid medium (PDA) or liquid medium (PDB) (Figure 1a-c). The *P. indica* strain is inoculated on PDA plate and activated for 14 days at 28 °C. The fermentation broth of *P. indica* is done with 50 mL ethyl acetate for 2 hours, and the organic phase is dried with anhydrous sodium sulfate after concentrated and steamed at 40-45 °C and dissolved in 10 mL of anhydrous ethanol. The organic phase is diluted 100 times with anhydrous ethanol for application (Chen *et al*., 2013). *P. indica* has the best growth under the condition of lower stirring speed and higher working volume (Kumar *et al*., 2011). Increasing the growth and subsequent consumption of vegetative cells by using a certain level of glucose would enhance sporulation of *P. indica* (Kumar *et al*., 2011).

The fermentation medium with soluble starch as carbon source has a high viscosity, so that the stirring speed is higher. In shake flask culture, *P. indica* grows either in the form of pellets or as a homogeneous mycelial suspension. In bioreactors, the rapid growth of *P. indica* led to carbon depletion, ultimately modulating earlier sporulation and shorter sporogenesis. The pH in the late logarithmic period of *P. indica* reduces to 5.5–6.0 because of acid metabolism after full utilization of glucose. As a result, no pH control is needed for cultivation of *P. indica* on a complex carbon source. Sugar-deficiency strategy and soybean meal could significantly increase the biomass of *P. indica* and shorten the time needed to maximize spore production (Kumar *et al*., 2011). The optimum germination rate of *Kelussia odoratissima* plants is under the condition of spore suspension of *P. indica* (Ghabooli *et al*., 2019).

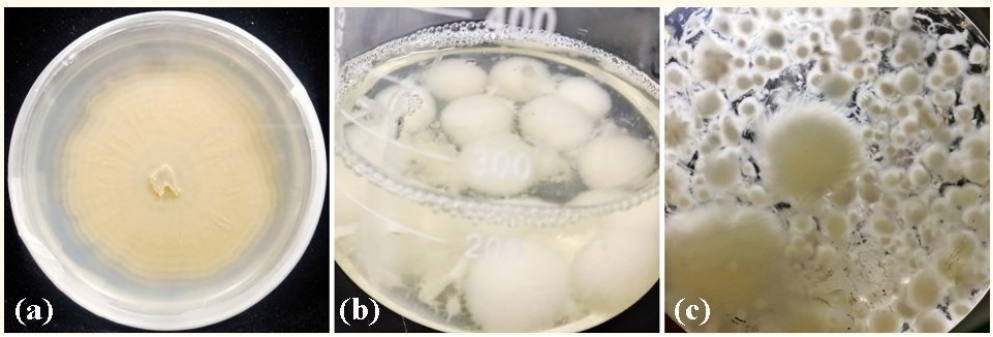

Figure 1. Culture of *Piriformospora indica* using potato glucose solid medium (a) and liquid medium (b and c)*Root colonization of Piriformospora indica and the recognition with plant roots*

Root colonization

In the process of fungal colonization, chlamydospores of *P. indica* first produce two branched germ tubes, which can induce the formation of appressorium (Jacobs *et al*., 2013). These appressorium's contact with plant roots to produce invasive nails, penetrate epidermis and cortical cells to complete the initial colonization process (Jacobs *et al*., 2013). *P. indica* colonization is mainly found in near root elongation zones and seldom at the top of the roots (Kumari *et al*., 2003), while its hyphae are not found in the aerial part of plants at any growth stage and colonization process (Kumari *et al*., 2003). The colonization pattern of the fungus is closely associated with the maturity degree of cells of roots (Boller *et al*., 2009; Unnikumar *et al*., 2013). In roots, a number of spores with pear shape are found in roots (Figure 2a-b).

Qiang *et al*. (2012) found that during the lethal stage of *P. indica* spores' colonization, spores inhibited the transduction of endoplasmic reticulum stress signals in *Arabidopsis*, and eventually led to a caspase-dependent cell death mediated by vacuoles. It implies that endoplasmic reticulum disfunction in roots trigger by *P. indica* is a strategy of microbes to kill plant cells.

When the *PiTam1* gene cloned from *P. indica* was silenced, the expression of auxin decreased, and the colonization of *P. indica* in barley roots decreased, suggesting that auxin might be a promoter in the colonization of *P. indica* (Hilbert *et al*., 2012). Furthermore, ethylene signals are required for the symbiosis and colonization of *P. indica*, as reported by Khatabi *et al*. (2012), which found that ethylene enhanced the fungal colonization in roots of *Arabidopsis thaliana*, and the damage in ethylene signaling pathway could reduce the fungal colonization. During the interaction between plants and *P. indica*, 926 proteins with signal peptides were secreted, whilst 543 proteins were used as effector factors (Rafiqi *et al*., 2013). In addition, *P. indica* also stimulates root cytokinin concentrations during fungal colonization (Camehl *et al*., 2010). These results fully demonstrate that in host colonization of *P. indica*, phytohormones from both hosts and *P. indica* are stimulated to accelerate root colonization. However, more studies are needed to be conducted to analyze the changes in phytohormones in the process of *P. indica* colonization.

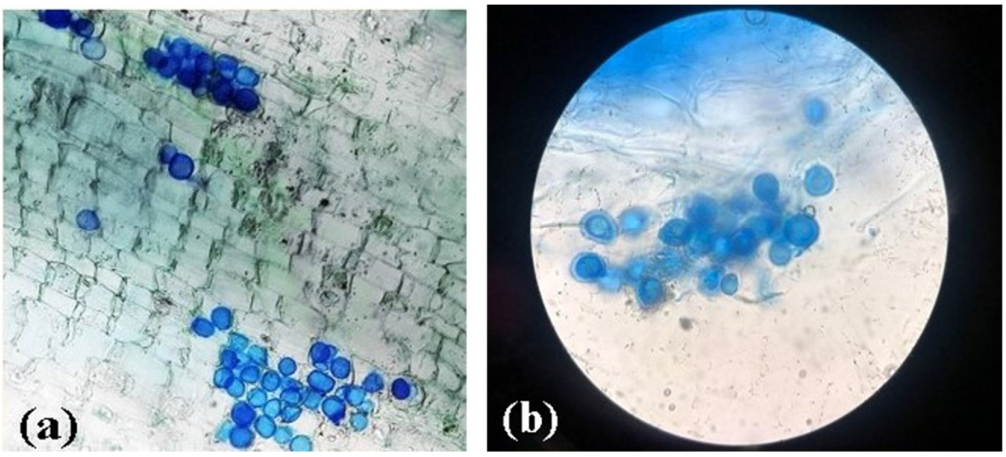

Figure 2. Root colonization of *Piriformospora indica* in trifoliate orange (a) and white clover (b)

Host recognition and signal responses

The recognition between *P. indica* and plant roots has signal responses. A series of host responses to symbiotic fungi are based on an efficient immune system within plants (Boller *et al*., 2009). Intracellular colonization of *P. indica* stimulates mode-triggered immunity (PTI) (Khatabi *et al*., 2012), which could be modulated by phytohormones, including ethylene, salicylic acid (SA) and jasmonic acid (JA) (Khatabi *et al*., 2012). The combination of both ethylene and JA blocks SA accumulation, whereas promotes the colonization

of *P. indica* (Khatabi *et al*., 2012). The inactivation of some components in the ethylene pathway seriously destroys the colonization process of *P. indica* (Camehl *et al*., 2013). The fungus relies on JA inhibiting initial immune responses (Camehl *et al*., 2013). Salicylic acid thioglycoside also inhibits the growth of mycelia, while the weak ethylene signals promote the hyphal growth (Varma *et al*., 2001). During the interaction with roots, it is very important to maintain low ascorbic acid levels in roots, because *P. indica* is not stable in mutant plants with ascorbic acid reductase (Vadassery *et al*., 2009). After the fungal colonization, the levels of gibberellin, auxin and other hormones in plants also increased in varying degrees (Schäfer *et al*., 2009; Camehl *et al*., 2013). With symbiosis between the endophytic fungus *P. indica* and ethylene signal mutants of *Arabidopsis thaliana*, cellotriose was identified as a novel chemical mediator taking part in communications of *P. indica* and plants (Johnson *et al*., 2009). Schäfer *et al*. (2009) found that ethylene played a key role in the root colonization, and it did not cause slight up-regulation of defense responsive genes but inhibit the expression of SA signal pathway-related genes after the colonization of *P. indica*. On the contrary, it could inhibit the expression of SA-signal-associated genes (Schäfer *et al*., 2009).

The intracellular Ca^{2+} concentration increased significantly in the early stage of the fungal colonization of roots (Vadassery *et al*., 2010). The extract from cell walls of *P. indica* also caused similar reactions in *Arabidopsis thaliana*. It is suggested that Ca^{2+} may be an early signal of the fungal root colonization. *P. indica* induces lectin protein kinase in plant roots, which is a crucial factor in perception and recognition for fungal colonization (Verma *et al*., 2017).

Roles of Piriformospora indica in host plants

Promotion of plant growth

Earlier studies have demonstrated positive plant growth promotion by *P. indica* in host plants (Figure 3; Table 1) (Barazani *et al*., 2007; Sharma *et al*., 2014). The inoculation with *P. indica* increased the biomass of marine algal, and the metabolites of glutamic acid and succinic in marine algae were raised and lipid structure was improved (Bhatnagar *et al*., 2019). In a medicinal plant *Aloe vera*, inoculated with *P. indica* significantly promoted biomass, plant height, root length, root number, number of buds, and chlorophyll levels. *P. indica* dramatically mitigates the damage on shoot and root biomass of infested cucumber plants (Atia *et al*., 2020). Similarly, the positive effect of the root endophyte on plant growth was reported in pistachio, beans, acacia, mung beans, and peas (Varma *et al*., 2001). The expression of miRNA and their target genes in orchid roots was induced by *P. indica*, whilst the target genes were involved in hormone signals, cell wall metabolism and regulatory transcription factors (Ye *et al*., 2014), suggesting that promotion of plant growth by *P. indica* is associated with improving the miRNA model. In addition, the fungus heavily stimulates the accumulation of auxin, andrographolide, and hexadecanoic acid, which is associated with plant growth (Bhatnagar *et al*., 2019). The root endophyte also promoted plant growth in *Arabidopsis thaliana* and tobacco seedlings, which is related with the more accumulation of nitrogen and the induced expression levels of nitrate reductase and amylo degrading enzyme genes (Sherameti *et al*., 2008).

The culture filtrate of *P. indica* also stimulated plant growth (Varma *et al*., 2001; Sharma *et al*., 2013; Bagde *et al*., 2014). Bagde *et al.* (2014) used the culture filtrate of *P. indica* into *Aristolochia elegans*, and found the enhancement in root number and length, plant height, leaf number, and plant biomass. Culture filtrate of *P. indica* also promoted hairy root biomass of *Vigna mungo* (Bagde *et al*., 2014) and plant growth of *Artemisia annua* (Sharma *et al*., 2013), flax plants (Kumar *et al*., 2012), and sunflower (Bagde *et al*., 2011).

In the early stage of the root endophyte colonization, intracellular Ca^{2+} concentrations were elevated in response to plant growth improvement (Vadassery *et al*., 2010). Additionally, gene expression analysis of *Chinese cabbage* induced by *P. indica* revealed that *P. indica* played an important role in inducing genes involved in transporting carbohydrate metabolism, hormone signaling, cell wall metabolism and root formation (Lee *et*

al., 2011). Further, auxin induction is involved in the plant growth improvement by *P. indica*, as seen in *Chinese cabbage* with higher auxin levels from the induced expression of genes related to cell wall acidification and auxin transporter. Similarly, *P. indica* could interact with orthology of sugar beet Hs1 PRO-1 2 to control the early growth of tobacco seedlings. Phosphorus transporter genes in *P. indica* promote plant phosphorus uptake, because these gene expressions are induced in external mycelium (Yadav *et al*., 2011).

In a word, *P. indica* strongly promotes plant growth, which is associated with nutrients, auxin, miRNA model, expressions of specific genes, phytoremediator, immunomodulatory, and bio-herbicide (Khalid *et al*., 2019).

Table 1. The effects of *Piriformospora indica* on nutrient acquisition, abiotic stress tolerance, and disease resistance of part host plants

Plants	Responses on nutrient acquisition	Responses on abiotic stress	Responses on disease resistance	References
Arabidopsis thaliana	P↑	nitrate reductase↑; ascorbic acid↑	verticillium wilt↓	Sherameti *et al*., 2005
Brassica napus	N↑; P↑; S↑; Zn↑; Mn↑	antioxidant enzyme activities↑	incidence of root rot↓	Chen *et al*., 2013
Chinese cabbage	P↑	antioxidant enzyme activities↑; chlorophyll and thylakoid protein degradation↓	incidence of black spot↓	Sun *et al*., 2010
Cucumis sativus	–	–	root knot nematode↓	Atia *et al*., 2019
Hordeum vulgare	–	activity of ascorbic acid reductase↑; ascorbic acid↑; glutathione↑	incidence of root rot↓; powdery mildew fungus↓	Waller *et al*., 2005; Ghaffari *et al*., 2019
Nicotiana tabacum	N↑; P↑; Zn↑	chlorophyll↑; indole acetic acid↑; catalase↑; superoxide dismutase↑	incidence of herbivorous insects↓	Abdelaziz *et al*., 2019
Triticum aestivum	N↑; P↑	ascorbic acid↑; acid reductase↑; catalase↑; deoxyascorbic↑; glutathione reductase↑; peroxidase↑	fusarium↓	Baltruschat *et al*., 2008
Zea mays	P↑	deoxyascorbic acid reductase↑; glutathione↑ oxidative potential↑; abscisic acid↑; auxin↑; cytokinins↑; salicylic acid↑	incidence of root rot↓	Stein *et al*., 2008; Zhang *et al*., 2018

Note: The symbol "↑"and "↓" means the significant increase and the significant decrease in the parameter after *Piriformospora indica* colonization. The symbol "-" means that author did not analyse the change in the parameter

Increase of nutrient acquisition

There are generally two ways for plants to absorb P directly from roots and from symbiotic fungi. In the interaction between *P. indica* and barley, the fungus increased the maximum use of P (Waller *et al*., 2005) and accelerated the transformation from insoluble and agglutinated or complex forms of P into soluble P in soil (Singh *et al*., 2000). In the colonization of barley, tobacco and mung bean, *P. indica* did not increase the content of N and P in plants, although it promoted the plant growth of plants (Achatz *et al*., 2010). In contrast, the fungal colonization significantly changed N, P and K concentrations in lentils and chickpeas these plants (Nautiyal *et al*., 2010). *P. indica* heavily increased phosphatase activities and expression levels of *ACP5* in roots of *Brassica napus* to accelerate P acquisition (Wu *et al*., 2018). In addition, the colonization of *P. indica* could

alleviate the damage caused by Fe and Cu deficit in sugarcane (Table 1) (Gosal *et al*., 2010). The root endophyte colonization increased N, P, S, Zn, and Mg levels in rapeseed and N, P, and Zn concentrations in tomato (Sarma *et al*., 2011; Chen *et al*., 2013). *P. indica* also increased the content of radiolabeled P in *Arabidopsis thaliana* and *Anthurium* spp., which originates from the up-regulated expression of P transporter (Sherameti *et al*., 2008; Lin *et al*., 2019). Fungal *PiMgT1* transporter of Mg was involved in the absorption of Mg in wheat exposed to Zn deficiency stress (Prasad *et al*., 2018). In addition, *P. indica* up-regulated NADH-dependent nitrate reductase gene expressions to promote N acquisition (Serfling *et al*., 2007).

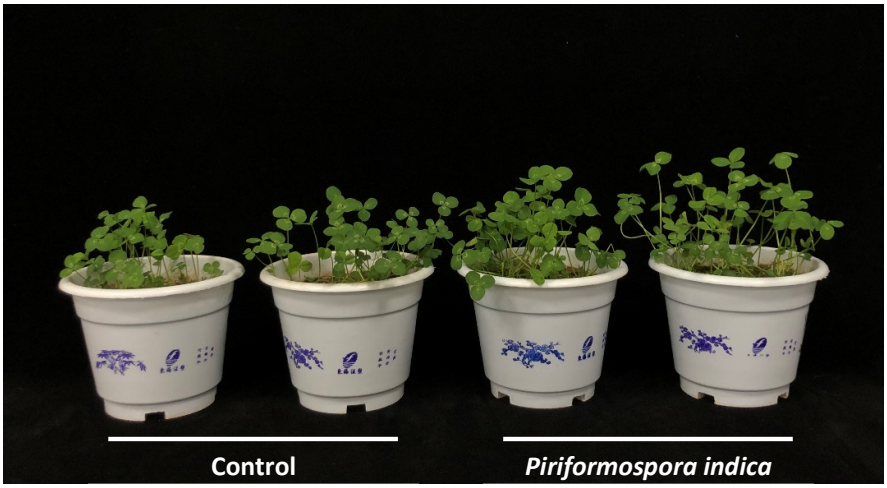

Figure 3. A positive effect of *Piriformospora indica* on plant growth response of white clover after 26 days of the fungal inoculation

Enhancement of stress tolerance

In addition to plant growth and nutrient acquisition, *P. indica* also enhances stress tolerance of plants (Table 1). For example, *P. indica* enhanced drought tolerance of Chinese cabbage through increasing antioxidant enzyme activity, reducing chlorophyll and thylakoid protein degradation, and alleviating photosynthetic efficiency decline (Sun *et al*., 2010). Similarly, chlorophyll, indole acetic acid, catalase, superoxide dismutase and *LeNHX1* expression by colonization of *P. indica* were increased under salt stress (Abdelaziz *et al*., 2019). Under adverse conditions, *P. indica* increased the levels of photosynthesis, antioxidant defense systems and energy transfer proteins to tolerate the negative effect (Ghabooli *et al*., 2013). In barley, the activity of ascorbic acid reductase and the content of both ascorbic acid and glutathione increased after inoculation with *P. indica* under salt stress (Waller *et al*., 2005). Likewise, colonization of *P. indica* conferred superior drought adaptation of barley through enhanced activity of both photosystems and electron transfer chains and promoted accumulation of photorespiration proteins (Ghaffari *et al*., 2019). Under drought stress, *P. indica* stimulated gene expression in maize associated with hormone functions, including abscisic acid, auxin, salicylic acid and cytokinins (Zhang *et al*., 2018). Colonization of *P. indica* in wheat roots could alleviate membrane lipid peroxidation and fatty acid desaturation in leaf tissues under salt stress, and increased the content of ascorbic acid and the activity of ascorbic acid peroxidase, catalase, deoxyascorbic acid reductase and glutathione reductase in root tissues under salt stress (Baltruschat *et al*., 2008). The colonization of *P. indica* in *Centella asiatica* exposed to P deficit showed a positive effect through the increase in acid/alkaline phosphatase activity, total phenolics and superoxide dismutase activity (Jisha *et al*., 2019). In rape, Chen *et al.* (2013) reported that the root endophyte had a significant improvement in drought tolerance, because of lower plasma membrane permeability and higher proline content, antioxidant enzyme activities, and drought-related

responsive gene expressions. Moreover, an increase in antioxidant enzymes activities were found in *Medicago truncatula* by the endophyte under high salinity conditions (Li *et al*., 2019). Sarma *et al.* (2018) analyzed the effect of *P. indica* on tomato, and revealed that the fungus increased tomato plant growth yield and prevented the occurrence of fusarium wilt. Under the conditions of water deficit and drought, protein analysis of barley leaves inoculated with *P. indica* showed that the fungus increased the level of proteins involved in photosynthesis, antioxidant defense system and energy transmission (Ghabooli *et al*., 2013). *P. indica* inoculation increased the expression level of a RNA editing factor to enhance the plant's capability to salinity stress (Hassani *et al*., 2019).

Recently, Ghaffari *et al.* (2018) used the proteomic and metabolomic technologies to reveal the molecular basis of barley plants inoculated with *P. indica*. They found that the fungal inoculation could readjust plant metabolites and proteome, redistributes resources of host plants, and keeps aquaporin protein activity in response to water deficit, which confers better drought tolerance in plants.

Enhancement of disease resistance

P. indica not only improved the ability of plants to resist root diseases, but also improve the ability of plants to resist leaf diseases (Table 1) (Ghaffari *et al*., 2018). Rabiey *et al.* (2015) found that *P. indica* had no direct antagonistic effect on fusarium *in vitro*, but the wheat inoculated with *P. indica* found few signs of brown rot, compared with uninoculated wheat. The phenomenon was related with higher ascorbic acid and glutathione levels and better antioxidant enzyme activities (Rabiey *et al*., 2015). The colonization of *P. indica* in cucumber also significantly increased chlorophyll levels and alleviated the negative effects of root knot nematode (*Meloidogyne incognita*) on photosynthesis (Atia *et al*., 2020). The inoculation of *P. indica* significantly improved the resistance of maize to root rot, because of greater activities of catalase, glutathione reductase, glutathione transferase and superoxide dismutase (Kumar *et al*., 2009). Nassimi and Taheri (2017) found that *P. indica* increased the rice biomass and also delayed the infection process of *R. solani*, resulting in the decrease of sheath blight severity. *P. indica* also increased the pH in the axoplast of leaves in barley infected by powdery mildew fungus, which further induced the expression levels of defensive genes and the accumulation of resistance-related substances in plants (Felle *et al*., 2009). Therefore, the systemic resistance induced by *P. indica* may be related to the change of plastid pH.

P. indica could significantly reduce the expansion of verticillium wilt in *Arabidopsis thaliana in vitro* after verticillium wilt infection, and other signals of spores were involved in the defense of Arabidopsis against verticillium wilt (Sun *et al*., 2010). Trzewik *et al.* (2020) successfully use *P. indica* as a possibility of biological protection against *Phytophthora* in rhododendron plants. *P. indica* induced resistance to powdery mildew in mutants of JA signaling pathway, and did not induce the transcript levels of defensive genes involved in SA signaling pathways (Stein *et al*., 2008).

Promotion of bioactive substance accumulation

P. indica has a critical role in promoting bioactive substance accumulation of plants. The aristolochic acid concentrations in leaves of *Aristolochia elegans* were heavily induced by the fungal culture filtrate (Bagde *et al*., 2014), indicating an important role in accelerating bioactive substances. In *Artemisia annua*, the culture filtrate of *P. indica* comparatively increased artemisinin levels, an antimalarial compound (Sharma *et al*., 2013). The increase in artemisinin content caused by inoculation with *P. indica* may be due to the enhanced biosynthesis of the artemisinin after the colonization of the fungus *P. indica*. Other studies also indicated that *P. indica* increased lignan concentrations of *Vigna mungo* (Kumar *et al*., 2012) and oil contents of sunflower (Bagde *et al*., 2014). These results confirmed the positive influence of *P. indica* in bioactive substance accumulation of plants. However, more studies will need to clarify the causal relationship.

Piriformospora indica: a potential fertilizer in the future of agriculture

It was found that *P. indica* and *Bacillus brevis* could promote black jelly beans more obviously than *P. indica* alone (Anith *et al*., 2015). PDB is a commonly used medium for *P. indica* fermentation, whereas *B. pumilus* cannot grow in PDB, which limits the development of *P. indica* compound fertilizer with *B. pumilus*. *P. indica* fertilizer can be fermented by coconut water, and thus economical coconut water can be used to co-culture *P. indica* and *B. pumilus*, providing a possibility for mass production of *P. indica* (Tripathi *et al*., 2015).

Compared with liquid bacteria, *P. indica* fertilizer has incomparable advantages in spore efficacy, cell viability, and transportation. Tripathi *et al.* (2015) explored *P. indica* as a carrier of biological agents. They used the mixture of *P. indica* and talc powder at 5% mass ratio as carriers that is the most stable and effective. The mixture maintains 108 CFU/g at 30 ℃, and the validity period of spores can be extended to 180 days.

Varma *et al.* (2001) observed that the fermentation broth of *P. indica* was filtered through double-layer gauze to obtain *P. indica* mycelium, and magnesium sulfite was used as a carrier mixed with mycelium using 2% of the mass ratio, whose activity was the most effective and stable.

To obtain the commercial production of spores, Varma (2017) proposed that *P. indica* was cultivated in a 7 L batch bioreactor with nanostructured materials "zinc oxide" as nanoembedded fungus. Such protocol can provide 9.25×10^9 spores/mL with zinc oxide nanorods. This will confer a kind of innovative technique for providing a path for agricultural application

Conclusions

P. indica has a wide range of colonization in plants and shows various functions, such as promoting plant growth, enhancing stress tolerance and disease resistance, and accelerating nutrient acquisition and bioactive substance accumulation (Figure 4). In addition, the effective colonization of *P. indica* improves rhizosphere microorganisms' activity to reduce the toxicity of heavy metal-contaminated soil, or induces expression of resistant genes (Zhu *et al*., 2019). Compared with mycorrhizal fungi, *P. indica* has obvious advantages in culture *in vitro*. Based on this, more and more attention has been paid to *P. indica* in sustainable agriculture and environment. For example, Liu *et al.* (2019) established a co-culture system between *P. indica* and oncidium plants in G10 medium *in vitro* to enhance plant growth. Li *et al.* (2019) used *P. indica* plugs and chlamydospora suspension into rooting medium of banana seedlings in vitro and found the fungal strongly stimulated plant growth and shorten the rooting time. This result provides a valuable idea for shortening the time of tissue culture in plants that are difficult to rooting. However, due to lack of understanding molecular mechanisms in the interaction of *P. indica* and plants, the application of *P. indica* has still been limited (Shahollari *et al*., 2007). *P. indica* can be used as potential biological hardening agent for endangered micro propagated plants such as *Picrorhiza kurroa* to improve the survival rate (Das *et al*., 2017).

Additionally, growth conditions and environments influence the interaction between *P. indica* and plants and the production and application of commercial inoculants, which need to be further studied. The fungus can be transported over long distances as inoculants, and can be produced in large quantities by economically viable methods such as nano-method, promising vigor growth for crops grown in poor soils. *P. indica* also enhances disease resistance of plants, which would reduce the use of pesticides, thereby, mitigating the pressure on the environment and improving food safety (Lou *et al*., 2007).

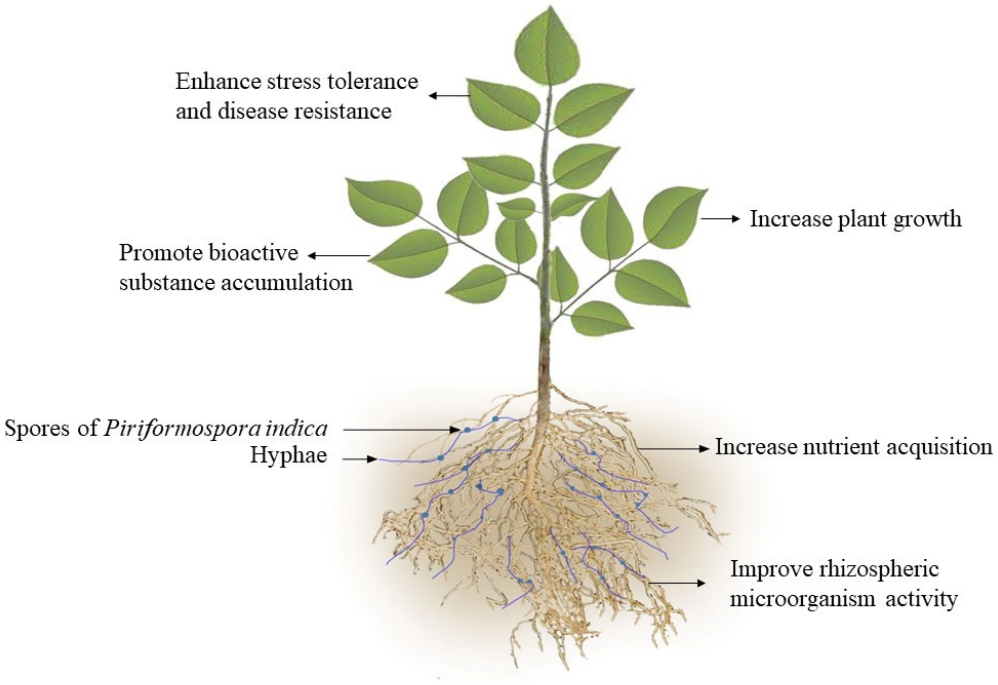

Figure 4. The positive effects of *Piriformospora indica* on plants

An important characteristic of *P. indica* is the comparative improvement of plant growth in various crops, while the underlying mechanisms are still unclear, relative to mycorrhizal fungi (He *et al*., 2019, 2020; Wu *et al*., 2019; Zhang *et al*., 2020; Zou *et al*., 2019). On the other hand, the fungus also enhances the tolerance of abiotic stress in plants, whereas the signal mechanisms and pathways of the increased antioxidant defense systems remain to be elucidated. The interaction between *P. indica* and other bacteria/fungi in improving plant growth still needs to be studied.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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