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Piriformospora Indica in Combination with N and P Alleviates the Effect of Drought and Heavy Metals Ions

Batool A[*1], Mingfang Zhang[1], Ahmed Mahmoud[1,2] and Ahsan M[3]

[1]Laboratory of Germplasm Innovation and Molecular Breeding, Institute of Vegetable Science, Zhejiang University, China
[2]Horticulture Research Institute, Agriculture Research Center, Egypt
[3]Department of Horticultural Sciences Faculty of Agriculture and Environment, The Islamia University of Bahawalpur Pakistan, Pakistan

[*]**Corresponding author:** Batool A, Laboratory of Germplasm Innovation and Molecular Breeding, Institute of Vegetable Science, Zhejiang University, Hangzhou 310058, China. Tel: 03037942868, Email: semee_uca@yahoo.com

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Abstract

The root dwelling fungus *Piriformospora indica* has potential application in pursuit of physiological and agronomical traits for crop improvement. Due to its unique advantage to be cultivated axenically, *P. indica* is frequently used as bio fertilizer, bio protector and bio regulator in many crop plants. Previous studies put forward the vital significance and biology behind *P. indica* exploitation as biological agent in agriculture sector to improve stress tolerance, water absorption, mineral uptake, photosynthesis, plant growth and development and yield as well. This review is focusing on the importance of *P. indica* root colonization, the role of endophyte in improving nutrient uptake and the survival of colonized crops under water deficient conditions. *P. indica* mitigates the influence of drought and heavy metal ions, improved Nitrogen (N) and Phosphorous (P) uptake in plants with enhanced physiological attributes such as plant growth promotion, adventitious roots elongation, early flowering, and alteration in secondary metabolites and also yield related traits.

Keywords: Stress, Environment, Abiotic, Microbes, Soil, Crop Production

List of Abbreviations:

P.I: *Piriformospora Indica*
P: Phosphorus
N: Nitrogen

Introduction

In natural ecosystems, plants continuously interact with a range of soil microorganisms. Numerous useful microbes with outstanding results have been studied, and among all microbes root endophytic fungi, *Piriformospora indica* is most important due to its potential to offer abundant opportunities in modern horticulture. In plant microbe interaction mutualistic symbiosis is beneficial for both microbes and plants [3]. In plants, *Piriformospora indica* is reported to inhibit ethylene signaling where, it encourages plant growth and development [21]. The mutual interaction of *P. indica* with plant roots has vital importance in crop improvement [52]. Also, it lessens the use of fertilizers, mends average crop production, changes the assembly of secondary metabolites and also offers resistance against biotic and abiotic stress conditions [11]. In colonized crop plants, it deliberates various physiological traits like nutrient and water uptake, photosynthetic rate, early flowering, improved seed germination with good crop production, biomass and ultimately fitness to the environment especially in nutrient deficient soils [42, 1]. *P. indica* emulates several benefits to the host plants for both vegetative and reproductive growth attributes with improved stress resistance [47]. The endophyte improved plant performance in all reverences including improved crop growth, productivity and enhanced root proliferation by indole-3-acetic acid production which in turn results into a better nutrient attaining capacity. Additionally, *P. indica* is able to extract, mobilize and transport N, P and some minor elements in the aerial plant parts. Through colonization *P. indica* mobilizes micronutrients from soil and make them easily accessible to the plants [16]. The root-associated microorganisms, such as endophytic fungi, can inactivate, remove or reduce harmful environmental pollutants [48, 32]. Exploring plants and the relevant microorganisms to remove pollutants provides a cost-effective and favorable technology.

***Piriformospora Indica* and Host Plant Tolerance to the Heavy Metal Ions:** Heavy metals being apparent environmental pollutants are getting huge attention at present. For normal growth and metabolic activities plants exposed to heavy metals adopt numerous mechanisms to detoxify their adverse effects. Plants classified heavy metals within cell by adopting two strategies to guard their organs from adverse effects of heavy metal, the first one is the restricted uptake of heavy metals and the second one is tolerance mechanism with little accumulation of heavy metals [8]. However, in second strategy, the restriction of heavy metal absorption is related to rhizospheric microorganism (fungus). The sources of environmental heavy metal contamination are combustion, mining extraction and industrial sewages. Arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), nickel (Ni), chromium (Cr) and aluminum (Al) all are the principal heavy metals that causes toxicity to both fauna and flora in soil. Most of these heavy metals are easily stocked in plants, enter in food chain and get transferred to the humans, causing serious diseases and disorders [4]. Regardless of the fact that some heavy metals such as iron (Fe), copper (Cu), selenium (Se) and zinc (Zn) are indispensable in small concentrations, but their accumulation at higher levels may become very toxic to the environment [3]. Fungus can lessen heavy metal ions uptake into the plant cells with the help of phenolic compounds [15]. *P. indica* colonized *Arabidopsis* and wheat plants perform better under heavy metal stress [29]. Endophytic fungus retains several degradation pathways, such as chelation systems and metal sequestration by which they increase host heavy metal tolerance and support host survival in contaminated soils [40]. In symbiosis, either fungi induce direct resistance in host plants to deal with the heavy metal toxicity or indirectly it improves tolerance by improving water and mineral nutrient uptake in plants, alternating root morphology and increasing shoot biomass. Endophytic fungi are the crucial components of root microflora in metal contaminated ecosystems [27]. *P. indica* possesses an excellent capacity to immobilize heavy metals in plant roots and can improve host plant tolerance to the heavy metal ions that is promising in phytoremediation [34]. Endophytic fungi *P. indica* supported phytoremediation that is economically effective and also environment friendly.

***Piriformospora Indica* Colonization and Host Plant Tolerance to Biotic and Abiotic Stresses:** Drought is the most important abiotic stress that hurdles plant growth, survival and productivity. It is a startling check on crop development and productivity throughout the world that causes decrease in rate of CO_2 assimilation, disruption of pigments and failing of photosynthesis [20]. The antioxidant defense mechanism, reactive oxygen species (ROS), superoxide radicals, hydrogen peroxide and hydroxyl radicals are affected by drought. According to the Food Security Information Network and Global Network Against Crises (2020) [11] there are more than 700 million people in the world suffering from food insecurity. The endophytic fungi *P. indica* can improve stress tolerance and cope with the adverse effects of drought through different mechanisms. For physiological and biochemical improvement in plants these mechanisms comprise of antioxidant enzyme enhancement, better phytohormonal levels, osmotic adjustment, modification in the biofilm production that results in improved nutrient uptake, better gas exchange capacity and good water use efficiency.

P. indica colonized seedlings exhibited faster and stronger up regulation of drought resistance related phospholipase HAT, Dδ, and CBL1 in addition to the improved activities of ROS scavenging enzymes present in cell membranes, mitochondria, peroxisomes and chloroplasts [39]. Likewise, to mimic drought stress when *P. indica* colonized Chinese cabbage plants were exposed to 20% polyethylene glycol (PEG) the colonized plants tolerate well due to the improved activities of superoxide dismutases, catalases and peroxidases in leaves, whereas, the non-colonized plants could not survive [41]. Furthermore, fungus delayed drought induced decline in photosynthesis, the degradation of thylakoid proteins, chlorophyll and the stress marker malondialdehyde. The proteome analysis of *P. indica* colonized barley leaves under water scarcity and well treated conditions revealed that *P. indica* improved energy transport levels and photosynthetic system along with antioxidant defense protein system [13]. Based on the plant species, root colonization with *P. indica* improved water stress tolerance. Drought resistance response of *P. indica* colonized plants was related to the increase in plastid localized proteins in the leaves of these colonized plants [41]. The endophyte inoculated *Arabidopsis*, Chinese cabbage and strawberry plants were recovered soon from drought [38]. Additionally, the gene expression profile of drought responsive genes such as *ANAC072*, *DREB2A*, *CBL1* and *RD29A* were positively modified in the leaves of *P. indica* colonized plants under drought stress conditions [41]. Drought tolerance revealed as higher endogenous proline level which in turn led to the improved tolerance to osmotic stress in endophyte colonized plants when compared with the non-colonized plants [41]. *H. vulgare* plants pre-inoculated with *P. indica* exhibited better enzymatic and non-enzymatic antioxidant levels result in the enhanced drought stress resistance [7].

***Piriformospora Indica*: Plant Mutualistic Interaction and Nitrogen Uptake:** Nitrogen (N) deficiency in soil has been reported to restrict plant growth and development [49]. Plants obtain N as nitrate in some species by fixing it with the help of rhizobium [10]. Sherameti et al. (2005) [37] reported that the co-cultivation of *Nicotiana tobaccum* and *Arabidopsis* seedlings with *P. indica* is accompanied by the transfer of N from agar plates to the seedlings aerial parts by the activation of NADH dependent nitrate reductase (NR) enzyme. *P. indica* activates NR enzyme which in turn plays important role in nitrate acquisition. Though, the stimulation of nitrate integration by *P. indica* is there for growth promotion. Enzymes related to the nutrient uptake are highly affected by drought, resulting in dropping nitrate uptake from the soil [19]. Reduction in transpiration rate due to scarcity of water decreased plant nutrients absorption and their utilization efficiency [33]. To limit plant growth and development the main feature of any symbiotic plant microbe interactions is its capacity to assimilate Nitrogen (N), Phosphorus (P) and micronutrients [16]. Root colonization with *P. indica* improved the uptake and assimilation of Nitrogen in plants. Furthermore, Bajaj et al. (2014) [5] observed the increase in biomass and secondary metabolites production in Turmeric after colonization with *P.indica* (Table 1). Fungus interaction with *Arabidopsis* roots is accompanied by the utilization of N from the environment [29]. *P. indica* mediates nitrate uptake from the soil, the fungus enhances NADH dependent nitrate reductase (Nia2) movement in the roots of *Arabidopsis* and tobacco which helps to improve nitrate uptake and its absorption from the soil [37]. In addition to, Sherameti et al. (2005) [37] identified a cis-regulatory element in Nia2 promoter that is essential for Nia2 gene expression and is targeted by BHL1 transcription factor. Similarly, the fungus improved the expression of starch degrading enzyme, SEX1, which encodes a glucan water dikinase by cis-regulatory element [37].

In plants and endophytic mutual interaction, the endophyte is known to improve Nitrogen uptake from roots [50]. In fungus colonized *Brassica napus* the positive agronomic and yield characteristics are linked with the accumulation of Nitrogen and Phosphorus along with Sulphur, Potassium and Zinc. *P. indica* mediated growth promotion in mung bean is positively correlated (3 to 4 fold) to the significant uptake of N 1.6 fold, P and K 1.4 fold from soil under glass house and field conditions [22]. Collectively, all studies supported that *P. indica* affects primary metabolism in roots positively by improving nutrients supply to the plant.

Plants	Role of *Piriformospora indica*	References
Potato	Stimulates tuberization	Upadhyaya et al. 2013
Soybean	Promotes plant growth charactericts	Varma et al. 2012
Mung bean	Stimulates plant growth	Varma et al. 2012
Pea	Improves growth and yield	Varma et al. 2012
Chick pea	Promotes growth and production	Varma et al. 2012
Holy basil	Increased biomass and secondary metabolites production	Das et al. 2012
Turmeric	Increased biomass and secondary metabolites production	Bajaj et al. 2014

Table 1: Role of *Piriformospora indica* in plant nutrient uptake, growth and yield attributes

***Piriformospora Indica*: Plant Mutualistic Interaction and Phosphorus Uptake:** For proper plant growth and development P is one of the most essential mineral nutrients that constitutes upto 0.5% dry weight of the cell [6]. P is required in substantial amounts to perform various structural, regulatory and energy transfer roles. *P. indica* is an efficient phosphate mobilizer that produces phosphatase enzyme which splits phosphate ester bonds of insoluble organic phosphates, polyphosphates and different organic acids which in turn solubilize the insoluble polyphosphates [26]. *Piriformospora indica* grows on various organic, inorganic and polyphosphates P sources and plays its part as an active P solubilizer apart from being as P mobilizer [36].

Plants obtain P from soil either by direct uptake with the help of its own transporters and also by using mycorrhizal links [50]. The uptake of radiolabelled P from culture medium and its translocation to the host is facilitated by *P. indica*. Shahollari et al. (2005) [35] reported that in *Arabidopsis* seedlings the phosphate uptake is 2 to 3 times improved by using *P. indica*. *P. indica* arbitrated growth promotion in *Arabidopsis* linked with massive uptake of radio labeled P from the growth medium. The uptake of Phosphorus and its transport is stimulated in endophyte colonized roots of maize [50]. There was no significant change observed in the biomass at seedling stage, however, the difference in biomass was significant between colonized plants grown in Phosphorus deprived conditions, and non-colonized plants grown in P enriched conditions at lateral stages. *P. indica* improved biomass more proficiently in P deficient conditions as compared to P enriched conditions. Thus, *P. indica* could be a good candidate for use in sustainable agriculture for better yield especially in P deficient soils [23]. The characterization of high affinity phosphate transporters was done through several plants and fungal species including *Medicago truncatula, Arobidopsis thaliana*, *Solanum tuberosum*, *Lycopersicon esculentum*, *Saccharomyce scerevisiae* and *Neurospora crassa* [18]. Phosphorus deficiency delays crop production all over the world, *P. indica* gives considerable amount of acid phosphatases that enables host plant to access sufficient amount of condensed, insoluble or complex forms of phosphate reserves in the soil. During P solublization in soil both acid and alkaline phosphatases take part, acid phosphatases involved in P uptake confined with cell membrane and plasma membrane of mycorrhizal roots while, alkaline phosphatases are present only in hyphal membrane of fungal symbiont [12].

The endophyte *P. indica* has uncovered the importance of phosphate transporter gene (PiPT) for the transport of P into the host plants [50] but the molecular mechanism underlying *P. indica* mediated phosphate transport in the host plants is not very clear. According to Yadav et al. (2010) [50] in *Zea mays* plants *P. indica* is involved in phosphate transfer, and the involvement of *PiPT* gene is indirect. Agreeing to the reports already discussed, (Table 2) Yadav et al. (2010) [50] reported that *Zea mays* plants colonized with wild type *P. indica* increased biomass and phosphate contents as compared to the non-colonized and KD-*PiPT P. indica* colonized ones. These results point towards the importance of *P. indica* in improving *Zea mays* biomass and yield. Furthermore, after colonization with *P. indica* phosphate transporter (PiPT) gene, transport phosphates from soil to the plant [28]. PiPT gene exhibits 12-trans membrane helices linked by a large hydrophilic loop in the middle and belongs to the high affinity phosphate transporter family. PiPT has 1815 bps and the activity of the enzyme is localized to the external hyphae of *P. indica* colonized host roots [28]. Upon colonization the fungus tempts phosphate transporters of *A. thaliana* e.g. PhT1-1 to PhT1-5 to enhance P utilization from soil by the host plant [25]. The fungus colonized mung bean plants had significantly higher amount of Nitrogen, Potassium and Phosphorus as compared to the untreated plants (Table 2, Kumar et al. 2012). In host plants, the exact mechanism of *P. indica* in phosphate transfer and spread is unclear and it is presumed that this process might occur at plant-fungus interaction. The whole process involves two transporters: the first to allow efflux of phosphate from fungus and second to facilitate phosphate uptake by the plant (Rausch and Bucher 2002). *P. indica* effectively increased the growth of *Linum album* by improving antioxidant machinery [22]. *P. indica* colonized *Piper nigrum* plants exhibited more noumber of leaves per plant and maximum fresh weight [2]. The growth of colonized plants at 40 mM phosphate was equal to the non-colonized plants grown at 400 mM indicated that *P. indica* can mimic plant growth even at10-fold with low P. Bi-compartment assay for 32P transportation revealed that fungal hyphae transport P to the host plants in P deprived conditions but unable to transfer more Phosphorus in P enriched conditions. Hence, it is concluded that *P. indica* has an ability to increase the biomass of maize plants especially under low phosphate conditions.

Growth promotion (biomass-g/plant)				
Host plants	Effect	*-Pi*	*+Pi*	References
Rice (*Oryza sativa*)	Root length	11.9±1.5	18.1±1.3	Jogawat et al. (2013)
	Shoot length	13.1±0.5	15.3±0.3	
	Fresh weight	81.1±3.1	105.0±1.5	
	Dry weight	22.0±1.3	31.6±0.7	
Coat buttons (*Tridax procumbens*)	Shoot length (cm)	57.3±1	0 73.0±0.9	Das et al. (2013)
	Root length (cm)	30.5±0.9	35.4±0.8	
	Root (FW)	6.9±0.1	11.2±0.5	
	Shoot (FW)	19.0±0.4	15.0±1.2	
	Root dry weight	2.4±0.3	4.7±0.3	
	Shoot dry weight	6.8±0.3	9.2±0.3	
Mung bean (*Vigna mungo*)	Root dry weight	1.2±0.1	2.9±0.1	Kumar et al. (2012)
	Shoot dry weight	13.9±1.8	34.9± 2.1	
	No. of pods per plant	17.6±1.8	65.7±5.5	
	N-mg/g plant	5.6±0.4	8.8±0.3	
	P-mg/g plant	3.1±0.4	4.2±0.2	
Sugarcane (*Saccharum officinarum*)	Cane no./clump	8.1	15.9*	Varma et al. (2012)
	Cane height (cm)	179.0	191.0	
	Sugar content (Brix)	18.2	21.4	
Mustard (*Brassica campestris* ssp. *Chinensis*)	Shoot fresh weight (mg)	20.5±2.1	45.5±2.6	Lee et al. (2011)
Black Pepper	Root (FW)	9.0	11.0*	Anith et al. (2011)
Chinese cabbage	Root (FW-mg)	8.5±1.2	20.4±1.1	Sun et al. (2010)
Arabidopsis thaliana	Biomass (mg)/seedling	38.5±5.2	59.5±2.6	Shahollari et al. (2007)
	Seed weight (mg)/plant	154.9±3.3	188.3±5.1	Sherameti et al. (2005)

* Significant <P 0.05%

Table 2: *Piriformospora. indica* induced growth promoting effect in different plants

Conclusion

P. indica is one of the most promising root endophyte for practical application under field conditions. It has a wide host range and is highly beneficial for host plants to stand in moderate to extreme biotic and abiotic stress conditions. In addition to drought, salinity, oxidative and heavy metal stress the endophyte confers resistance to the soil pathogens. Another significant feature of *P. indica* is its aptitude to colonize a variety of unrelated host plants that led to the promotion of this endophyte as a reputed bio fertilizer, bio modulator and as a biological control agent. Even though, in nutrient deficient soil *P. indica* can improve N and P uptake into the host plants, while the plants provide Carbon to the fungus. This review offers an exclusive exploration on the role of *Piriformospora indica* in the uptake of Nitrogen and Phosphorus, with heavy metal ions and drought stress resistance for good plant growth and yield related attributes.

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