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Piriformospora indica mycorrhization increases grain yield by accelerating early development of barley plants

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Root colonization by the basidiomycete fungus *Piriformospora indica* induces host plant tolerance against abiotic and biotic stress, and enhances growth and yield. As *P. indica* has a broad host range, it has been established as a model system to study beneficial plant-microbe interactions. Moreover, its properties led to the assumption that *P. indica* shows potential for application in crop plant production. Therefore, possible mechanisms of *P. indica* improving host plant yield were tested in outdoor experiments: Induction of higher grain yield in barley was independent of elevated pathogen levels and independent of different phosphate fertilization levels. In contrast to the arbuscular mycorrhiza fungus *Glomus mosseae* total phosphate contents of host plant roots and shoots were not significantly affected by *P. indica*. Analysis of plant development and yield parameters indicated that positive effects of *P. indica* on grain yield are due to accelerated growth of barley plants early in development.

The wide majority of plant roots in natural ecosystems is associated with fungi, which very often play an important role for the host plants' fitness.[1] The widespread arbuscular mycorrhizal (AM) symbiosis formed by fungi of the phylum Glomeromycota is mainly characterized by providing phosphate to their host plant in exchange for carbohydrates.[2,3] Fungi of the order Sebacinales also form beneficial interactions with plant roots and *Piriformospora indica* is the best-studied example of this group.[4] This endophyte was originally identified in the rhizosphere of shrubs in the Indian Thar desert,[5] but it turned out that the fungus colonizes roots of a very broad range of mono- and dicotyledonous plants,[6] including major crop plants.[7-9] Like other mutualistic endophytes, *P. indica* colonizes roots in an asymptomatic manner[10] and promotes growth in several tested plant species.[6,11,12] The root endophyte, moreover, enhances yield in barley and tomato and increases in both plants resistance against biotic stresses,[7,9] suggesting that application in agri- and horticulture could be successful.

P. indica-induced Higher Grain Yield is Independent of Low Soil P Levels and Elevated Pathogen Pressure

As AM fungi improve the uptake of P into roots of host plants,[13] and as *P. indica* was shown to induce an enhanced uptake of P into the cotyledons of Arabidopsis grown on artificial medium,[14] it was tested whether *P. indica*-mediated yield increase depends on P and N levels of the soil.[15] Surprisingly, the endophytes' positive influence on barley grain yield was independent of the factor 'fertilization', e.g., was present also when P and N supply were high, indicating that *P. indica*-induced yield increase does not depend on low P or N supply.

In addition, barley plants colonized by the AM fungus *G. mosseae* showed an increased P content of shoots and roots, while such an effect was not observed for *P. indica*.[15] This is in line with earlier experiments showing that *P. indica* is not inducing a significant increase of leaf P and N content in the dicotyledonous plant *Nicotiana attenuata*,[16] but different from recent results obtained with maize.[17] *P. indica* effects on yield—at least in the crop plant barley—are therefore not dependent on low P supply, do not lead to an elevated total P or N content at the end of plant development, and are mechanistically different from effects of AM fungi.

Which mechanism is the basis of enhanced growth and grain yield of *P. indica* colonized barley plants, if not plant nutrition? *P. indica*-induced enhanced defence-readiness of the host plant[7-9] could result in a lower metabolic cost of pathogen defence, thereby increasing yield. This hypothesis could not be confirmed: elevated pathogen pressure by applying the necrotrophic fungus Fusarium to the soil did not result in a significantly changed yield effect of *P. indica*.[15] Therefore, enhanced biotic stress resistance alone can't explain the observed grain yield increase.

P. indica-induced Higher Grain Yield is Based on Host Plant Reprogramming Early in Development

P. indica-induced higher grain yield is based on a higher number of ears per plant. The number of ears, in turn, is a result of the number of tillers formed by the plant. It is therefore conceivable that *P. indica* must play an important role before and during tiller formation.

Indeed, *P. indica* colonization leads to initial advantages of the host plant: Barley root growth is strongly induced between two and three weeks after inoculation,[7,18,19] and further development of colonized roots is reflected by higher expression levels of developmentally regulated genes.[20] Faster root development could be initially induced by auxin-like substances produced by the fungus,[21] while complex regulatory processes in the host tissue already take place during the first days of colonization as revealed by transcriptome analysis of *P. indica* colonized barley roots.[22]

We suggest that increased root growth results in improved acquisition of water and nutrients in the early phase of the symbiotic interaction. This would be in line with results of Yadav et al.[17] who detected higher phosphate contents in maize plants three weeks after *P. indica* inoculation. This initial advantage of colonized plants could be the basis for a more vigorous development of the shoot (as observed from 2.5 weeks after inoculation in barley) and an improved physiological status leading to the initiation of more tillers (which starts from about 4–5 weeks after inoculation). In addition, the observed enhanced photosynthetic rates of colonized plants[15] contribute to this improved status.

The initial advantages of colonized plants are not only leading to the formation of more tillers, but also result in faster development, e.g., the observed earlier emergence of ears.[15] Interestingly, *P. indica*-induced growth promotion is not resulting in higher straw yield to grain yield ratio or taller plants, nor higher contents of mineral nutrients at the end of the vegetative phase, indicating that the host plant is able to control its growth pattern and development during the symbiotic relationship. However, improved early nutrient status promoting tiller formation, and an earlier resource allocation to the ears for the production of grains together with a higher capacity for producing assimilates leads to a higher grain yield.

Taken together, *P. indica* is not inducing an uncoordinated vigorous growth at all stages of host plant development, but is improving host plant growth and faster development at an early stage, providing the basis for higher grain yield at the time of harvest. Further functional characterization of specifically expressed genes in the *P. indica*-barley interaction is expected to provide a better understanding of host reprogramming that eventually leads to enhanced grain yield. In turn, these genes could be exploited in new strategies improving crop grain yield in the future.

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