

Indigenous Arbuscular Mycorrhizal Performance

Subjects: Plant Sciences

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It is difficult to assess the function of indigenous microorganisms interacting with plants in the environment. The function can be evaluated by using mutants of host plants that are unable to express the function. The function of arbuscular mycorrhizal fungi (AMF) in roots can be assessed by using symbiotic mutant of *Lotus japonicus* that do not form arbuscules, which are their nutrient exchange organs.

Keywords: arbuscular mycorrhiza ; mycorrhizal mutant, ; *Lotus japonicus*, ; indigenous fungi, ; phosphate, ; growth promotion, ; inoculation

1. Introduction

Most plants, including many economically important crops, are usually colonized with arbuscular mycorrhiza fungi (AMF) in the subphylum Glomeromycotina ^[1] in the fields. AMF colonize roots to obtain carbon sources and develop extraradical hyphae that absorb mineral nutrients from the soil and transfer them to the host plants ^[2]. AMF that have achieved a symbiosis that is sufficient to amplify their own biomass often form spores in the soils and develop intraradical mycelia with many vesicles, although these hyphal morphologies depend on the AMF type ^{[3][4]}. These vegetative and reproductive AMF hyphal structures serve as a source of inoculation for colonizing different plant species in fields, because AMF generally lack strict host specificity; accordingly, the root is often co-colonized with multiple AMF species ^{[5][6]}.

2. AMF

To investigate the biological properties of each AMF species, many cultured lines have been established by isolating spores and inoculating a single spore in plants in a pot culture or axenic root organ culture ^[7]; however, they have been generated using only limited species ^[8]. Inoculation studies of these lines in different plant species, with the exception of modern wheat or barley varieties ^{[9][10]}, have led to one well-recognized conclusion, i.e., AMF colonization is, in many cases, beneficial for plant nutrition and productivity (see the objective review ^[11]). Moreover, pot inoculation studies have shown that phosphate (P) uptake by the host plant is often improved and the P concentration in shoots is also increased in the mycorrhized condition compared with the non-mycorrhized condition ^[3]. This improvement in P uptake led to an expectation that AMF inoculants can be used as bio-fertilizers; i.e., that AMF inoculation in the field may enable farmers to decrease the amount of P fertilizers ^[12]. For example, inoculation of AMF can substantially reduce P fertilizer application to Welsh onions and lead to the achievement of a marketable yield under field conditions ^[13]; however, it remains debatable whether mycorrhizal fungi increase the transport of P to plants directly ^[14]. One way to utilize AM symbiosis in crop cultivation is to increase the propagules of indigenous AMF in the soil. The increased AMF propagules in the soil after the cultivation of host crops can improve the productivity of soybean crops in the following year compared with the former cultivation of non-host crops ^{[15][16][17]}. Accordingly, it is expected that P fertilization of the next-year cultivation can be reduced by half ^[18]. Another approach consists in inoculating the soil with exotic AMF culture lines. As mentioned above, it has been proven that AMF inoculation is useful in many cases, at least in pot tests under well-controlled environmental conditions. However, it is also true that not all inoculation tests provide positive results, even in pot experiments; some plant species clearly show a positive effect of the inoculation of AMF, while others show neutral or even negative effects of the same AMF ^[19]. The outcome of these mycorrhization approaches is considered to be context dependent ^[20]; i.e., the type of AMF, plant type, growth condition, growth stage, soil abiotic (nutritional and physical) and biotic (indigenous AMF and soil microbes) properties, etc., affect the performance of mycorrhizas ^{[21][22][23]}.

Whether the AMF inoculation strategy is effective or not remains a matter of debate ^{[8][11][24]}. Usually, the soil contains a large amount of AMF propagules ^{[25][26]}. In addition, it has been suggested that indigenous AMF are better adapted to the local edaphic conditions than are exotic AMF and are, thus, better able to promote plant growth ^{[27][28]}. In fact, there are few examples of increased crop yields after AMF inoculation in the field ^[29]. However, it is possible that the performance

of indigenous AMF is severely decreased in some soils because of various changes in the soil management history (e.g., excessive tillage, sterilization, and fertilization) [30][31]. Therefore, if the low performance of indigenous AMF could be investigated in advance, the inoculation of exotic AMF could also be effective [32].

To the best of our knowledge, there is no method for evaluating the performance of indigenous AMF. In this study, we developed a method to evaluate the performance of indigenous AMF in promoting plant growth in pot culture. We used a mycorrhizal mutant of *Lotus japonicus*, MG-20, which is a model plant of legumes. The mutant line has a nonsense mutation in the *STR* (*stunted arbuscule*) gene, which encodes an ABC transporter [33]. *STR* is thought to be implicated in lipid transfer from plants to AMF, as assessed using genetic analyses [34][35][36][37]. Recent genome analyses have revealed that AMFs are dependent on plants for lipid synthesis, which is essential for the establishment of symbiosis [38][39]. Thus, in the *str* mutant of *L. japonicus*, *Medicago truncatula*, and rice (*Oryza sativa*), the early root-colonization stage looks normal, but the branching of arbuscules, which are intracellular symbiotic fungal structures that play a central role in mycorrhizal function, is severely inhibited (*stunted arbuscule: str*) [33][40][41]. The expression of mycorrhiza-specific P and ammonium transporter genes in the *L. japonicus str* mutant is severely inhibited compared with wild-type (WT) plants [33], suggesting the attenuation of a broad range of mycorrhizal functions in this mutant. From a different perspective, if the growth of WT plants under the presence of AMF is similar to that of *str*, the effect of colonization with indigenous AMF under the assay condition is not expressed (i.e., low performance). Thus, we examined the growth ratios of WT and *str* mycorrhizal mutant using 24 soils. Furthermore, we investigated whether an AMF inoculum was effective in promoting plant growth in soils that were assessed as having low indigenous mycorrhizal performance.

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