**Varietal differences in the growth responses of rice to an arbuscular mycorrhizal fungus under natural upland conditions**

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**ABSTRACT**

Seedlings of three rice (Oryza sativa L.) varieties (one indica, ARC5955; and two japonica, Nipponbare and Koshihikari) with or without pre-colonization by the arbuscular mycorrhizal fungus *Funneliformis mosseae* were transplanted into an upland field and grown to maturity. Pre-colonization had no effect on the yield of Nipponbare or Koshihikari. However, pre-colonized ARC5955 exhibited a strong tendency toward increased yield, which was accompanied by increases in the percentage of ripened grain and the 1000-grain weight. The rice roots were also colonized by indigenous arbuscular mycorrhizal fungi in the field, but these had only limited effects on shoot biomass and grain yields. We speculate that *F. mosseae* may have exhibited priority effects, allowing it to dominate the rice roots. There was no significant difference in the contents of most mineral elements in the shoots of pre-colonized ARC5955 at harvest, indicating that some other factor is responsible for the observed yield increase.

**Abbreviations:** AM, arbuscular mycorrhiza or arbuscular mycorrhizal; MGR, mycorrhizal growth response

**Introduction**

There is currently an interest in not only elucidating the molecular mechanisms of symbiosis between rice (Oryza sativa L.) and arbuscular mycorrhizal (AM) fungi through laboratory experiments\(^1,2\) but also promoting the growth and increasing the yield of rice in the field through colonization with AM fungi. Although AM fungi are present in paddy wetlands,\(^3,4\) their colonization rate decreases with increased development of aerenchyma in the roots.\(^5\) Therefore, here we cultivated rice plants in an upland region to assess the effects of AM fungi on rice growth, like field experiments using other crops.\(^6-8\)

As a first step toward identifying quantitative trait loci (QTLs), which are important for producing a positive growth response and yield increase in rice, we grew seedlings that had or had not previously been inoculated with the AM fungus *Funneliformis mosseae* (formerly *Glomus mosseae*)\(^9,10\) for 4 weeks in a glasshouse, and looked for a combination of rice varieties that showed high and low growth responses to the fungus.\(^11\) Among the NIAS Global Rice Core Collection,\(^12\) the indica variety ARC5955 showed the strongest positive growth response to colonization by *F. mosseae*, whereas the japonica varieties Nipponbare and Koshihikari exhibited only low response.\(^12\) Furthermore, although seedlings of ARC5955 and Nipponbare exhibited similar changes in their mineral contents following colonization, only ARC5955 experienced growth stimulation, with a particularly large increase in P content.\(^11\)

Therefore, in the present study, we used pre-colonized seedlings of these three rice varieties that were grown to maturity. For field experiments, large amounts of inoculum are often needed.\(^6,7\) However, most of them can be saved through pre-colonization and transplantation of the seedlings. To our knowledge, this is the first trial to evaluate the effects of pre-colonization of AM fungus on the growth and yield of rice under natural upland conditions.

**Results and discussion**

In each year of the experiment, we applied less basal dressing than is standard (N, P, and K were c. 40%, 20%, and 20% of the conventional amounts, respectively) and no top dressing to the field to allow the effects of AM colonization to be clearly observed. Four 4-week-old seedlings that were at the 4–5 leaf stages and had not yet started to tiller were transplanted into each plot. The colonization percentages were between 3 and 12% for the pre-inoculated seedlings, whereas no fungal structures were observed in the control seedlings. At this point, the shoot biomass (mg) per plant of non-inoculated controls and that of pre-colonized seedlings were 110.4 ± 10.2 and 127.0 ± 6.7, respectively, for Nipponbare, 86.0 ± 4.1 and 106.7 ± 5.8, respectively, for Koshihikari, and 89.8 ± 21.1 and 141.3 ± 34.0, respectively, for ARC5955. We defined mycorrhizal growth...
response (MGR) by the following equation:

\[
MGR \% = \frac{100 \times (DW_{AM} - DW_{NM})}{DW_{NM}}
\]

where \(DW_{AM}\) and \(DW_{NM}\) were shoot dry weights of AM and non-mycorrhizal seedlings, respectively. The MGR values (%) for Nipponbare, Koshihikari, and ARC5955 were 15.0, 24.1, and 57.3, respectively. The hill spacing was c. 50 cm × 50 cm, to minimize the cross-infection of AM fungi between the roots of different plants, and pre-colonized seedlings were transplanted into a separate bed from non-inoculated control seedlings to prevent the migration of AM fungi (Fig. 1). The mean ambient temperatures during the field experiments are shown in Fig. 2. In 2011, it became hot earlier than average, resulting in a large temperature difference between 2011 and 2012. However, according to the Ministry of Agriculture, Forestry and Fisheries of Japan (http://www.maff.go.jp/j/study/suito_sakugara/h2403/pdf/ref_data2-3.pdf), the rice yield indexes in Aichi Prefecture were the same for both years (101).

Nipponbare and Koshihikari form nonglutinous short grains, while ARC5955 forms nonglutinous long grains. Although all of these are intrinsically paddy rice varieties, we grew them in upland fields in the present experiment to remove the effect of waterlogging from the activities of the fungi. Table 1 shows the yield and yield components of the three varieties in 2011 and 2012. For Nipponbare, the pre-colonization of \(F. mosseae\) had no significant effect on any of the yield and yield components, and there was no interaction between pre-colonization and year; pre-colonization also had a variable effect on grain yield, decreasing it in 2011 and increasing it in 2012. For Koshihikari, the growth was unstable, with all of the yield and yield components showing significant year effects, possibly indicating that the upland and nutrient-poor conditions were particularly unsuitable for this variety; however, it is noteworthy that the pre-colonization of \(F. mosseae\) also had no significant effects on this variety, with a variable effect on grain yield again being observed. By contrast, ARC5955 that had been pre-colonized by the AM fungus showed a strong tendency toward increased yield in both 2011 and 2012, although this effect was not statistically significant (\(p = 0.070\)). Furthermore, the 1000-grain weight of pre-colonized ARC5955 was significantly higher than the control. It is also noteworthy that the percentage of ripened grain was also higher in pre-colonized ARC5955, and this difference was marginally significant (\(p = 0.054\)). Therefore, the yield increase that was observed in pre-colonized ARC5955 was likely due to combined increases in the percentage of ripened grain and the 1000-grain weight.

During the cultivation period, the rice roots were also colonized by indigenous AM fungi in the field, resulting in the percentage of the root length colonized in non-inoculated controls being quite similar to that of pre-colonized plants (Table 2). Infection by indigenous fungi was also observed in 2013, although to a much lower extent than in 2011. The percentages of the root length colonized in non-inoculated controls and that of pre-colonized plants were 5.8 ± 1.2 and 16.8 ± 1.8, respectively, for Koshihikari, and 9.3 ± 1.2 and 23.5 ± 3.5, respectively, for ARC5955. Infection by indigenous AM fungi

**Figure 1.** Design of the field experiment conducted in 2011.

**Figure 2.** Mean ambient temperatures measured every 10 days at the experimental site at Nagoya during the cultivation periods. Solid and dashed lines represent 2011 and 2012, respectively. Source: Local Meteorological Observatory of Nagoya.
Values are means ± SD (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001; ns, not significant.

Table 2. Colonization of rice roots by arbuscular mycorrhizal (AM) fungi in 2011 at harvest. − and + indicate plants that were not inoculated and pre-inoculated with *Funneliformis mosseae*, respectively. Values are means ± SD (n = 3).
Therefore, we directly added the entire inoculant to the soil by
containing Kanuma soil. A soil inoculant of F. mosseae was
then transplanted individually into 40-mL cells in a plastic tray
varieties Nipponbare and Koshihikari were germinated and
Seeds of the indica rice variety ARC5955 and the japonica rice
Materials and methods

Table 3. Mineral contents (μg/g DW) of the aboveground parts of rice at harvest. — and + indicate plants that were not inoculated and pre-inoculated with Funneliformis mossae, respectively. Values are means ± SD (n = 3).

| Variety | F. mossae | N | P | K | Ca | Fe | S |
|---------|-----------|---|---|---|----|----|---|
| Nipponbare | — | 25567 ± 7315 | 1082 ± 135 | 17502 ± 2814 | 1894 ± 400 | 199 ± 26 | 1163 ± 250 |
| + | 25567 ± 8541 | 1038 ± 180 | 20609 ± 4043 | 1827 ± 261 | 192 ± 86 | 1141 ± 192 |
| Koshihikari | — | 26933 ± 2511 | 1153 ± 249 | 15930 ± 3509 | 2003 ± 238 | 168 ± 30 | 1423 ± 438 |
| + | 26400 ± 10739 | 1130 ± 491 | 13487 ± 5042 | 1669 ± 477 | 194 ± 118 | 1424 ± 180 |
| ARC5955 | — | 38800 ± 9111 | 1422 ± 334 | 15036 ± 2859 | 1697 ± 81 | 250 ± 70 | 1258 ± 185 |
| + | 29700 ± 3904 | 1234 ± 182 | 16494 ± 1422 | 2034 ± 257 | 301 ± 132 | 964 ± 151 |

Thus, the effects of the indigenous AM fungi again appeared to
be limited, even if they infected rice roots during the cultivation
period.

To understand why the shoot biomass and grain yield of
pre-colonized ARC5955 increased, we measured the shoot min-
neral contents using ICP-AES. This showed that the Zn content
was significantly lower in pre-colonized plants, while the other
10 elements remained at similar levels (Table 3). These results
are in contrast with increased contents of P and N by inocula-
tion of other crops with AM fungi.6,8 It is currently dif-
cult to explain why a reduced Zn content would increase the grain
yield, and so we cannot exclude the possibility that the different
growth responses of plants may have been caused by some
other factor, such as different plant hormone levels. Alterna-
tively, the yield increase may have simply been a secondary
effect of the excellent growth of ARC5955 seedlings due to
F. mosseae colonization.11-12 Indeed, there is an old saying in Japan,
“Nae Hansaku,” which means “If healthy seedlings are pre-
pared, good yield of rice grains is guaranteed by half.” There-
fore, a QTL analysis between ARC5955 and a japonica variety
may help to clarify the mechanism of yield increase by F. mos-
seae, which, in turn, may be effective for improving the yield of
good tasting japonica rice through the use of AM fungi in sus-
tainable agricultural systems.

Materials and methods

Seeds of the indica rice variety ARC5955 and the japonica rice
varieties Nipponbare and Koshihikari were germinated and
then transplanted individually into 40-mL cells in a plastic tray
containing Kanuma soil. A soil inoculant of F. mossae was a
kind gift from Idemitsu Kosan (Tokyo, Japan). We initially
found that the contaminating microorganisms in the
filtrate of a soil inoculant of F. mossae that had been passed through
38-μm mesh10 had no significant effect on plant growth. Therefore, we directly added the entire inoculant to the soil by
shaking 1 L of Kanuma soil (c. 420 g) and 45 mL (30 g) of the
F. mossae inoculant in a plastic bag and then adding 40 mL of
the mixture to each cell for pre-colonization. For the control,
40 mL of pure Kanuma soil was added to the cell. The seedlings
were grown in a cultivation room that was maintained at 28°C
under a 16 h day / 8 h night cycle at a light intensity of
204 μmol m⁻² s⁻¹ for 4 weeks. Watering was carried out as
required and the soil in each cell was supplemented twice dur-
ing the cultivation period with 20 mL of 0.5× modified Hoag-
land solution, which contained 100 μM phosphate.

The field experiments were carried out at the Higashiyama
Campus of Nagoya University, Aichi Prefecture, Japan (35° 1’
N, 137° 0’ E, 61 m a.s.l.) from 2011 to 2013. In 2011, we per-
formed the experiments in a fallow upland area that had been
covered with weeds in the previous year, whereas in 2012 and
2013, we used an upland area that had been converted from
flooded paddy fields. The land was plowed twice with a small
tilling machine, and basal dressing (N, P, K = 2.2, 1.7, 1.5 g
m⁻²) was applied as a 1:1 mixture (w/w) of a quick-acting com-
pound fertilizer (Toho Co., Osaka, Japan) and the coated fertil-
izer Hitomaki-kun (JA Aichi, Nagoya, Japan). We then
constructed beds that were 40 cm high × 1 m wide) consisted of four plants (Fig. 1). The plants
were grown until harvest at the end of October. Watering was
carried out as required and no top dressing was applied during
the cultivation period.

In 2011 and 2012, we harvested three and four hills, respectively,
in each plot. All panicles were counted and threshed by hand, and
filled and unfilled spikelets were separated and counted. After
oven-drying, we determined the 1000-grain weight and calculated
the grain yield for the filled unhulled grains. Unfortunately, no yield
measurements could be made in 2013 because two typhoons
occurred in September, severely damaging the rice plants.

In 2012, all of the stems and leaves on each of the sampled
hills were also oven-dried and combined with the unhulled
grains to determine shoot biomass. The combined materials were then pulverized with a small electric crusher and ground with a mortar and pestle. A portion of each ground sample was digested by heating it with HNO₃, allowing the inorganic element contents to be determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES; IRIS ICAP, Nippon Jarrell Ash, Tokyo, Japan). Another portion of the ground sample was digested by heating it with NaOH and K₂S₂O₅ and then neutralized with HCl, allowing the nitrate content to be determined spectrophotometrically, from which we calculated the nitrogen content.

In 2011 and 2013, portions of the roots were also collected at harvest, cleared with KOH, and stained with trypan blue, following which we determined the root length.

...We calculated the nitrogen content.

...the mycorrhizal fungal inoculants in the field revealed using molecular genetic tracing and measurement of yield components. New Phytol 2012; 194:810-22; PMID:22380845; http://dx.doi.org/10.1111/j.1469-8137.2012.04090.x

...of arbuscular mycorrhizal fungi (Rhizopagus clarus) increase yield of soybean and cotton under field conditions. Front Microbiol 2016; 7:720; PMID:27303367; http://dx.doi.org/10.3389/fmicb.2016.00720

...which we determined the root length colonization by AM fungi (%), as described by McGonigle et al. 24

...We analyzed the data statistically using analysis of variance (ANOVA).

**Disclosure of potential conflict of interest**

No potential conflicts of interest were disclosed.

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**In Japanese with English title.**