MYCORRHIZAL COLONISATION ENHANCE THE GROWTH AND NUTRIENT UPTAKE IN DIFFERENT CROPS GROWN IN GLASSHOUSE

PENGKOLONIAN MIKORIZA MENINGKATKAN PERTUMBUHAN DAN PENGAMBILAN NUTRISI PADA BERAGAM TANAMAN DI RUMAH KACA

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(Diterima: 23 Januari 2006, disetujui: 11 Juli 2006)

ABSTRACT

This study aimed to investigate mycorrhizal colonisation on plant growth and nutrient acquisitions on three crop species, i.e, soybean (Glycine max (L.) Merr cv. Willis), upland rice (Oryza sativa cv. Cirata), and maize (Zea mays cv. Marshall). These crops were inoculated with arbuscular mycorrhiza fungi (AMF) Glomus mosseae BEG 107 grown in a Luvisol (Calcareous soil, pH 7.3 in $CaCl_2$) under glasshouse conditions. Inoculated plants became mycorrhizal while control plants remained non−mycorrhizal. The AM colonisation rates were as high as 60%, 40%, 70% of the total root length, respectively, in soybean, upland rice, and maize. Mycorrhizal crop growth increased 7.5−folds, 4.5−folds, and 5.0−folds relative to non−mycorrhizal crop, respectively, in soybean, upland rice, and maize. Related to nutrient uptake, mycorrhizal colonisation increased P concentrations in shoot 1.6−folds, 3.2−folds, and 1.6−folds; and in root 1.9−folds, 1.9−folds, and 2.6−folds, respectively, in soybean, upland rice, and maize. However, it increased, decreased or were similar with other elements depended on elements and crop species. Applied mycorrhizal clearly contributed to enhance the growth and nutrient uptake of crop tested.

INTRODUCTION

Crops such as soybean, upland rice, and maize are important crops as a food crop in the worldwide. However P limitation is a common phenomenon in calcareous soil. As a consequence, it inhibits plant growth under this soil. Arbuscular mycorrhizal fungi (AMF) are essential components of soil biota; they can be found in nearly all ecolog−ical situations, both in natural ecosys−tem, particularly in those supporting crop communities with high species diversity, and in normal cropping sys−tem, especially managed with sustain−able practice (Gianinazzi and Sch epp, 1994).

Introducing mycorrhiza which are well known are able to enhance P uptake to plant by enlarge soil volume through its extraradical hyphae. Phosphorus is a key element obtained by plants through the symbiosis and the evidence to support is extensive (Smith and Read, 1997). However, the effective AMF are those producing the greatest benefit in terms of incr−eased P acquisition for the least cost expressed as C expenditure on mycorrhizas (Graham and Eissenstat, 1998). Because the beneficial effect of the AM symbiosis is multifaceted and does not result solely from improved P uptake (Jeffries et al., 2003), in terms of overall plant health and sustainability, therefore, the benefit of establishing an effective AM symbiosis is much wider and more long−lasting compared to the cost of P fertiliser, especially for a short period (Jeffries

MATERIAL AND METHODS

These experiments were conducted in glasshouse from early May to early August (soybean), mid September (upland rice), and late August to mid November (maize). All crops, i.e., soybean (Glycine max cv. Willis), upland rice (Oryza sativa cv. Cirata), and maize (Zea mays cv. Marshall) were grown in a calcareous soil (Luvisol, subsoil, C-horizon). The soil was partially sterilised (dry heated) two times at approximately 80° C for 24 h with one day of cooling at room temperature between heating periods. Inoculum used was Glomus mosseae (BEG 107). As basal nutrients applied were in the following concentrations and forms: 100 mg N (NH_4NO_3), 200 mg K (K_2SO_4), 100 mg Mg ($MgSO_4.7H_20$), 2 mg Fe (NH_4-Fe-citrate, 28% Fe), 10 mg Zn ($ZnSO_4.7H_20$), and 10 mg Cu ($CuSO_4.5H_20$) per kg dry soil in all crops. While P in soybean and upland rice were 30 and 50 mg P as $K(H_2P0_4)_2$, respectively, in AM and nonAM crops. However, in maize the same amount of P, 50 mg P as $K(H_2P0_4)_2$, was applied in AM and nonAM crops. The second fertiliser was applied one month after sowing for 100 mg N as NH_4NO_3 per kg dry soil both in soybean and upland rice and 5 g/L Fe as sequestreen Fe-EDDHA only in upland rice.

The soils was mixed with 10% inoculum and nutrients with water content of the soil was approximately 8%. The soil mass was 1.5 (soybean and upland rice) and 4.0 kg dry soil (maize) per pot. Soil bulk density was adjusted to 1.3 g cm^{-3}. A nonAM crops received a similar amount of inoculum sterilised with autoclave (120° C, 20 min) plus a filtrate (Blue Ribbon filter paper no. 5893, Schleicher & Sch ll, Dassel, Germany) of non-sterilised mycorrhizal inoculum.

The seeds were sterilised with 10% H_2O_2 for 2-5 min and then pre-germinated for 8-24 hours depend on crop species in saturated $CaSO_4$. Plants were watered daily and adjusted to around 20% (w/w). Soybean and upland rice were sown at the same time and retained with two plants per pot while maize was sown later with one crop per pot. The crops were harvested 62, 106, and 84 days after sowing by severing shoots from roots, respectively, in soybean, upland rice, and maize. Then, shoots were dried in an oven at 60° C for three days and weighed. Roots were rinsed to remove soil, cut into 1-cm fragments and thoroughly mixed. Representative fresh samples (1 g) were removed for determination of root AM colonisation. The remaining roots were dried and weighed.

Roots samples for determination of root colonisation were cleared with 10% (w/v) KOH and stained with 0.05% (v/v) trypan blue in lactic acid as described by Koske and Gemma (1989) and microscopically examined for percentage of root length colonised by mycorrhiza using the gridline-intersect method (Giovannetti and Mosse, 1980). Dried shoots and roots were ground in a grinding machine and 250 mg of plant material samples was ashed for 5 hours at 550° C in a muffle furnace, and the ash suspended in 1:30 (v/v) HCl for determination of mineral nutrients. P was determined colormetrically, K and Ca by flamephotometry and Mg, Zn, Cu, Mn and Fe by atomic absorption spectroscopy (AAS). Total C and N were measured by oxidation process

1997 (SPSS Inc., Chicago, USA). Significance of ANOVA: NS, *, **, and *** indicated for non-significant or significant at 5%, 1% and 0.1% levels, respectively.

RESULT AND DISCUSSION

Plant Growth and Infection Rate

Inoculated plants became mycorrhizal while control plants remained non-mycorrhizal. The mycorrhizal colonisation rates were higher than 72%, 47% and 80% of the total root length in soybean, upland rice and maize, respectively (Table 1).

Soybean plant without mycorrhiza showed severe manganese toxicity symptoms (necrotic spots on old leaves). These symptoms were less severe in mycorrhizal plants. The symptom was observed on the leaves of most plants and growth was drastically inhibited in these plants. This was indicated by 8.0-folds greater total biomass in AM plants relative to nonAM plants at harvest time. However, it was similar in root/shoot ratio for both plants. In upland rice, both AM and nonAM plants grew slowly in the first month and showed slightly N and Fe deficiency. In the following month, the plants grew better after applied with 100 mg N and 5 g/L Fe in both plants, but the tendency was greater in AM plants than in nonAM plants. This growth was drastically significant, which was indicated by 4.7-folds greater total biomass in AM plants relative to nonAM plants at the harvest time, 106 d after sowing. However, the plant size even in AM plants was quite small compared to the field situation. In maize, one month after sowing both plants indicated N and P deficiency but it was more severe in nonAM compared to AM plants, particularly P deficiency symptoms. AM plants grew higher and possessed more leaves than nonAM plants. At the harvest time, 83 d after sowing, plant size drastically enhanced in AM plants indicated by 3.0-folds greater plant height and 5.1-folds total biomass relative to nonAM plants drastically retarded in nonAM plants. Meanwhile, nonAM plants showed severely necrotic as indicated by dark brown in all leaves (Table 1).

Nutrient Concentration and Content

Mycorrhizal colonisation increased P concentrations in shoot 1.6-folds, 3.2-folds and 1.6-folds and

Table 1. Mycorrhizal Colonisation Effect to Colonisation Rate, Shoot, Root Total Biomass and Root/Rhoot in Soybean, Upland Rice and Maize

11	Colonisation Rate (%)	Shoot (g)	Root (g)	Total Biomass (g)	Root/Shoot	Plant Height (cm)
Soybean						
+AM	72	6.50	2.30	8.80	0.35	34
-AM	0	0.80	0.30	1.10	0.37	27
Upland rice						
+AM	47	1.50	1.30	2.80	0.87	49
-AM	0	0.50	0.10	0.60	0.20	29
Maize						
+AM	80	20.20	5.30	25.50	0.26	105
-AM	0	4.10	0.90	5.00	0.22	35

Table 2. Mycorrhizal Colonisation on Nutrient Concentration in Shoot (S) and Root (R) of Soybean Upland Rice and Maize Plants

	N	P		K		Ca		Mg		Zn		Cu		Mn		Fe	
	S	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
	mg.g^{-1}									µg.g^{-1}							
Soybean																	
+AM	12	1.3	1.5	17	11	11	13	5.1	10	56	97	8	59	129	398	68	-
-AM	34	0.8	0.8	21	17	12	12	6.0	15	86	111	14	66	282	982	10 9	-
Upland Rice																	
+AM	26	1.6	1.3	26	16	5.3	18	2.5	-	74	174	32	179	347	411	41	-
-AM	22	0.5	0.7	18	9	6.3	9	2.1	-	64	169	19	259	125	196	65	-
Maize																	
+AM	8	0.8	1.3	13	10	3.8	15	3.1	4.1	27	61	8	69	71	289	63	-
-AM	19	0.5	0.5	36	10	6.8	10	3.0	3.8	38	97	13	61	66	233	74	-

all nutrient uptake were a consequence of increase in plants growth (Tables 3). However all crops, either AM or nonAM plants, indicated strongly low P and N concentration in shoot according to optimal standard (Tables 2). In soybean, Mn concentration in shoot was above optimal standard (21−100 ppm) in both plants, but it was 2−folds greater in nonAM plants relative to AM plants. In contrast, AM plants in upland rice were higher 2.8−folds Mn concentration in shoot relative to nonAM plants, while it was similar to maize plants (Tables 2 and 3).

These results clearly demonstrated for the positive effect of mycorrhizal colonisation on plant growth by enhance particularly P uptake. Plant requirement on P and N however were insufficient based on P and N status in shoot of all crops species. Thus, under sub optimal nutrients availability caused strong inhibition in plant growth particularly when plants without mycorrhizal inoculation. However the mechanism of mycorrhiza contribution was different between crop species. In soybean, it has a strong correlation with Mn toxicity. Meanwhile soybean compared to other crop species is very susceptible to soil manganese toxicity. This specific problem has been observed in many studies (Kothari et al., 1991; Arines et al., 1992; Posta et

Table 3. Mycorrhizal Colonisation on Nutrient Content in Shoot (S) and Root (R) of Soybean, Upland Rice and Maize Plants

	N	P		K		Ca		Mg		Zn		Cu		Mn		Fe	
	S	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
	mg.pot^{-1}									µg.pot^{-1}							
Soybean																	
+AM	76	76	3.4	108	27	70	30	33	24	369	226	52	137	847	929	443	-
-AM	28	28	0.3	18	5	10	4	5	5	73	48	11	21	236	317	91	-
Upland Rice																	
+AM	40	2.4	1.6	40	21	8	23	4	-	113	224	51	232	534	531	64	-
-AM	10	0.2	0.1	9	1	3	1	1	-	31	20	9	30	60	22	31	-
Maize																	
+AM	158	15	6.7	265	53	77	80	63	22	550	326	153	364	1433	1533	1281	-
-AM	79	2	0.4	148	7	28	7	13	3	151	65	52	54	269	163	296	-

dilution of Mn in the plant tissue due to better P uptake and growth, (ii) altered redox conditions in the rhizosphere of AM plants, (iii) modified activity of micro−organisms in soil due to the AM fungus. This manganese toxicity was not identified in upland rice even in AM plants were greater Mn concentration relative to nonAM plants. In these plant species an internal channel system (aerenchyma) allows diffusion of oxygen from shoot to the roots and subsequent release into the rhizosphere (Trolldenier, 1988). Thus another mechanisme was suggested by two possibility explanations (i) under low P in soil in particular low light intensity, upland rice was highly independent on mycorrhizal colonisation, (ii) the slow rate of growth particularly under greenhouse conditions (sub tropic conditions which have low temperature and light intensity) was also the reason for this specific plant growth adaptation with mycorrhiza particularly during early growth. In maize, (i) the first reason was probably better P uptake, (ii) under low natural light exposure and temperature during end of summer season caused low photosynthetic rate which directly reduced the rate of growth. This slow growth rate was probably beneficial for the young host plants which were actively growing, thus was able to control partition of C translocation to mycorrhizal part. Plants with inherently faster growth rates are less likely than those with slow growth rates to produce excessive photosynthate, which might be advantageously diverted to the mycorrhizal symbiont, resulting in positive responses to mycorrhizal colonisation over a wide range of soil P levels (Smith and Read, 1997). Lambers and Poorter, (1992), Smith and Smith (1997) argue that the slow growth rate might also be less susceptible to C limitation under low irradiance, especially if they are also able to adjust C allocation to roots and shoot. Recently, B cking and Heyser (2003) indicated that the exchange process between the symbionts in a mycorrhiza were possibly linked and that P uptake and translocation by an ectomycorrhizal fungus is also regulated by carbohydrate supply from host plant. These results revealed that a beneficial of mycorrhiza acted with specific way and depended on crop species, however it showed strong correlation between nutrient status in soil and light exposure.

CONCLUSION

All crops tested in these experiments indicated a positively response with mycorrhizal fungus inoculation. This was indicated by the enhance in crop growth 4.7−8.0 folds and nutrient content, particularly P, 1.6 − 3.2 folds in mycorrhizal crops relative to non−mycorrhizal crops.

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