

Effects of co-inoculation with arbuscular mycorrhizal fungi and rhizobia on soybean growth as related to root architecture and availability of N and P

Xiurong Wang · Qiang Pan · Fengxian Chen ·
Xiaolong Yan · Hong Liao

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Abstract Soybean plants can form tripartite symbiotic associations with rhizobia and arbuscular mycorrhizal (AM) fungi, but little is known about effects of co-inoculation with rhizobia and AM fungi on plant growth, or their relationships to root architecture as well as nitrogen (N) and phosphorus (P) availability. In the present study, two soybean genotypes contrasting in root architecture were grown in a field experiment to evaluate relationships among soybean root architecture, AMF colonization, and nodulation under natural conditions. Additionally, a soil pot experiment in greenhouse was conducted to investigate the effects of co-inoculation with rhizobia and AM fungi on soybean growth, and uptake of N and P. Our results indicated that there was a complementary relationship between root architecture and AMF colonization in the field. The deep root soybean genotype had greater AMF colonization at low P, but better nodulation with high P supply than the shallow root genotype. A synergistic relationship dependent on N and P status exists between rhizobia and AM fungi on soybean growth. Co-inoculation with rhizobia and AM fungi significantly increased soybean growth under low P and/or low N conditions as indicated by increased shoot dry weight, along with plant N and P content. There were no significant effects of inoculation under adequate N and P conditions. Furthermore, the effects of co-inoculation were related to root architecture. The deep root genotype, HN112, benefited more from co-inoculation than the shallow root genotype, HN89. Our results elucidate new insights into the relationship between rhizobia, AM fungi,

and plant growth under limitation of multiple nutrients, and thereby provides a theoretical basis for application of co-inoculation in field-grown soybean.

Keywords Soybean · Arbuscular mycorrhizal fungi · Rhizobia · Co-inoculation

Introduction

Soybean (*Glycine max* (L.) Merr) originated in China, and is a major protein source in the human food chain and an important component of food supplements and high-quality animal feed (FAO 2003). Since soybean consumption has increased rapidly in recent years, more soybean production is needed to meet the increasing demands of the world market.

Nitrogen (N) and phosphorus (P) are equally very important nutrients for plant growth (Marschner 1995). However, N and P availability is generally low in arable soils, thus making them major limiting factors for crop growth and yield, especially in tropical and subtropical areas (Graham et al. 1982; Graham and Vance 2000; Hardarson and Atkins 2003). During the past 50 years, the widespread use of chemical fertilizers to supply N and P has had a substantial impact on food production, and has become a major input in crop production around the world (Tilman et al. 2002). However, further increases in N and P application are unlikely to be as effective at increasing yields. Today, only 30–50% of applied N fertilizer and 10–45% of P fertilizer are taken up by crops (Adesemoye and Kloeppe 2009; Garnett et al. 2009). In addition, the heavy use of chemical fertilizers in agriculture causes frequent deleterious environmental consequences, and has become a global concern (Bohloul et al. 1992; Vance 1997; Tilman et al. 2002). Therefore, developing nutrient-efficient varieties will likely

X. Wang · Q. Pan · F. Chen · X. Yan · H. Liao (✉)
Key Laboratory of Soil and Plant Nutrition in South China,
Ministry of Agriculture; Root Biology Center,
South China Agricultural University,
Guangzhou 510642, China
e-mail: hliao@scau.edu.cn

play a major role in increasing crop yield and would be in a more sustainable and economical approach to agriculture.

Understanding the mechanisms of crop nutrient efficiency is the basis from which to develop nutrient-efficient varieties. Till now, a number of potential adaptive mechanisms have been demonstrated to contribute to plant P efficiency, including changes in root morphology and architecture, and secretion of acid phosphatases into the rhizosphere (Raghothama 1999; Vance et al. 2003; Wang et al. 2009). Besides these beneficial plant traits, appropriate symbioses also contribute to plant nutrient efficiency. For example, biological N fixation plays an important role in legume N efficiency as well as associated crops in rotation and intercropping systems (Tang et al. 2005; Li et al. 2009; Salvagiotti et al. 2009). In addition, the roots of many plant species are naturally colonized by arbuscular mycorrhizal (AM) fungi, which enhance plant P efficiency and help plants grow well under relatively harsh conditions (Bethlenfalvay et al. 1985; Smith and Read 1997; Smith et al. 2009).

Furthermore, legumes like soybean can form tripartite symbiotic associations with nodule-inducing rhizobia and AM fungi simultaneously, which may benefit both P and N efficiency (Lisette et al. 2003). But inconsistent effects of co-inoculation with rhizobia and AM fungi have been reported for different crops (Barea and Azcon-Aguilar 1983; Cluett and Boucher 1983; Lisette et al. 2003). Co-inoculation of soybean roots with *Bradyrhizobium japonicum* 61-A-101 considerably enhanced colonization by the mycorrhizal fungus *Glomus mosseae*, and increased N and P uptake (Xie et al. 1995). Facilitative effects of co-inoculation with rhizobia and AM fungi were also found in faba bean (Li et al. 2004). In contrast, none or negative responses to co-inoculation have been reported in green gram (Saxena et al. 1997) and pea (Blilou et al. 1999). Moreover, it was reported that N and P interactions in field-grown soybean could affect root morphological and nodular traits (Kuang et al. 2005), and results from a soil pot experiment indicated that genotypic variation for AMF colonization was related to root architecture in soybean (Liu et al. 2008). To explain the contrary reports of effects of co-inoculation with rhizobia and AM fungi, we postulate that variation of N and P status and/or root traits among experiments might play a role.

In the present study, a field experiment was carried out for studying the relationships between soybean root architecture, AMF colonization, as well as nodulation under natural conditions. Furthermore, a greenhouse soil pot experiment was conducted at different N and P levels using two soybean genotypes contrasting in root architecture to investigate the effects of co-inoculation with rhizobia and AM fungi on soybean growth as well as N and P uptake.

Materials and methods

Materials

Two soybean (*Glycine max* (L.) Merr.) genotypes contrasting in P efficiency and root architecture were employed. They were a P-efficient genotype, HN89, with a shallow root system, and a P-inefficient genotype, HN112, with a deep root system as described by Zhao et al. (2004). The original inoculum of AM fungi was *G. mosseae*, grown on *Zea mays* L with MPN value as 300 propagules per gram of soil. The inoculants were a mix of the infected roots, spores and mycelium. The soybean rhizobium inoculant was *Bradyrhizobium sp.* BXYD3 with MPN value of 1×10^9 rhizobia per milliliter of liquid (Cheng et al. 2009).

Experimental design and growth conditions

There were two experiments in this study. The field experiment was carried out on a typical acidic red soil with low P and N content in Boluo County, Guangdong Province, P. R. China. Basic soil chemical properties are shown in Table 1. High P (120 kg ha^{-1}) and low P (40 kg ha^{-1}) treatments were applied as calcium superphosphate. Added as urea and KCl were 120 kg N ha^{-1} and 80 kg K ha^{-1} , respectively. All fertilizers were applied through band fertilization. Each treatment had four replicates in a randomized complete block design. Each plot had an area of 15 m^2 and the planting density was $30 \times 10 \text{ cm}$. The MPN values of indigenous AMF and rhizobia in the field soil were 2.31 propagules and 9.67×10^7 rhizobia per gram soil (Cao 2007; Huang 2007), respectively. Fifty days after planting, representative root samples were dug

Table 1 Basic chemical properties of the tested soils

Experiment	pH	Organic matter (g kg^{-1})	Total (g kg^{-1})			Available (mg kg^{-1})		
			N	P	K	N	P	K
Field	5.27	11.5	1.14	0.48	6.54	83.76	7.95	97.65
Greenhouse	5.57	12.98	1.17	0.46	15.5	85.56	14.32	112.12

The chemical analysis was performed with the standard methods as follows: pH value: 2.5:1 (water/soil) extraction; organic matter: $\text{K}_2\text{Cr}_2\text{O}_7\text{--H}_2\text{SO}_4$ digestion; total N content: Kjeldahl method; total P content: $\text{H}_2\text{SO}_4\text{--HClO}_4$ digestion; total K content: NaOH fusion; available N content: alkaline diffusion; available P content: Bray II method; available K content: 1 M neutral NH_4OAc extraction

from the soil, and were measured for root width and root depth as described later, and nodule number and nodule dry weight. Sub-samples from the root samples were taken for measuring AMF colonization. The shoots and roots were harvested separately and dried for determination of dry weight, and N and P content.

The soil pot experiment was conducted in the greenhouse of the Root Biology Center at the South China Agricultural University. The soil was a lateritic red soil with relatively low available N and P content as shown in Table 1. The soil was sieved with a 2 mm sieve, then mixed with sand in a 1:1 ratio by weight, and disinfected by sunlight. There were 16 treatments in total, including two P (HP, 100 mg P kg⁻¹ added as KH₂PO₄; LP, no P added), two N (HN, 500 mg N kg⁻¹ as KNO₃; LN, no N added), two AM fungi (+M, 100 g pot⁻¹ added with inoculants; -M, 100 g pot⁻¹ added with sterilized inoculants) and two rhizobium inoculation levels (+R, added with 50 mL rhizobia liquid; -R, added with 50 mL sterilized rhizobia liquid). Soybean seeds were surface disinfected and germinated for 36 h at 25°C. Germinated seedlings with 1–2 cm emerging radicals were transplanted to soil pots. The experiment was laid out as a randomized complete block design with four replicates. Each pot consisted of 4 kg of soil–sand mix covered with 1 cm silica sand. Sixty days after planting, shoots and roots were harvested separately as described above. Plants were watered with deionized water daily throughout the growth period. The greenhouse had natural light and an average temperature of 25°C.

Evaluation of root morphological and architectural traits

In the field experiment, a representative plant was sampled from each replicate. A square block of soil (40×40 cm) with the plant base in the center was dug to reach the end of tap root to get the complete plant root system (1 m was the maximum depth for the soil block if the depth of tap root was deeper than 1 m; Zhao et al. 2004). Root architecture was described by the ratio of root width to depth as described by Ao et al. (2010). Root width was measured as the widest width of lateral roots, and root depth was taken as the length of the tap root.

In the soil pot experiment, whole plant root systems were carefully washed out from the pots and cleaned before being scanned and the digital images were quantified with computer image analysis software (Win-Rhizo Pro, Régent Instruments, Québec, Canada) for measuring root morphological parameters, such as root length, root surface area, and root volume (Zhao et al. 2004).

Quantification of AMF colonization

Three first-order lateral roots were sub-sampled for staining in an AM assay. The roots were cleared by 10% KOH for

7 days, and stained with 5% ink–vinegar solution according to the procedure of Vierheilig et al. (1998). Total AMF colonization of roots was determined as the percentage of root length colonized by AM fungi using the intersection method.

Data analysis

All the parameters taken from the experiments were statistically analyzed by ANOVA with replicates using Excel 2000 software (Microsoft Corporation, 1985–2003) and SAS 8.1 (SAS Institute Inc., Cary, NC, USA) for multiple comparison analysis.

Results

Field experiment

In the field, the two soybean genotypes, HN89 and HN112, significantly differed in their growth and uptake for both N and P in both low and high P treatment levels (Table 2). HN89 had greater plant biomass and total N and P content than HN112 at both P levels. The relative plant biomasses of HN89 and HN112 at low P compared to high P were 0.99 and 0.52, respectively. According to the definition of P efficiency given by Lynch (1998), HN89 was a P-efficient genotype compared to HN112. Furthermore, the relative total P content of HN89 and HN112 at low P compared to high P were 0.92 and 0.89, respectively. This indicated that HN89 had a similar P acquisition capacity as HN112 under natural conditions in the field.

Using the ratio of root width/depth as an indicator for root architecture, HN89 had a larger ratio of root width/depth compared to HN112 at both low and high P levels (Table 2), confirming that HN89 had a shallower root architecture and HN112 had a deeper root architecture as reported by Zhao et al. (2004).

Soybean genotypes can be naturally inoculated by indigenous AM fungi and rhizobia in the field. AMF colonization of the roots of both soybean genotypes was significantly affected by P availability (Table 2). AMF colonization generally increased with decreasing P availability, particularly for HN112. The AMF colonization of HN112 increased from 39.74% at high P to 68.16% at low P, but only slight differences in HN89 were found (Table 2). There were significant effects of P availability on soybean nodulation in HN112 as indicated by nodule number and dry weight, but not in HN89. As shown in Table 2, HN112 had greater nodule number and dry weight at high P than at low P. This indicated that both AMF colonization and nodulation in the deep root genotype, HN112, were more responsive to P availability in the field.

Table 2 Effects of P availability on soybean growth, root architecture, AMF colonization, and nodulation in field

	<i>P</i> level	Genotype		<i>F</i> value		
		HN112	HN89	P	G	P×G
Plant biomass (g plant ⁻¹)	HP	15.47±2.10	30.26±5.02	1.27	28.16	1.07
	LP	7.99±0.78	29.93±4.20	ns	***	ns
Plant N content (mg plant ⁻¹)	HP	46.93±3.58	163.40±2.18	61.27	788.11	50.54
	LP	44.55±4.37	113.95±2.95	***	***	***
Plant P content (mg plant ⁻¹)	HP	33.09±2.51	56.22±8.69	0.72	21.44	0.01
	LP	29.52±1.92	51.50±3.07	ns	***	ns
Root width/root depth	HP	0.73±0.13	0.87±0.06	0.18	9.74	2.92
	LP	0.60±0.08	1.07±0.11	ns	**	ns
AMF colonization (%)	HP	39.74±4.02	46.93±1.57	19.47	1.03	8.14
	LP	68.16±3.73	53.03±5.90	***	ns	*
Nodule number (# plant ⁻¹)	HP	18.88±3.77	16.17±0.38	0.46	0.80	4.40
	LP	12.63±1.52	19.38±1.93	ns	ns	ns
Nodule dry weight (g plant ⁻¹)	HP	0.23±0.01	0.23±0.04	3.97	1.20	1.94
	LP	0.15±0.03	0.21±0.01	ns	ns	ns

Values in the table are the means of four replicates with standard error

ns not significant

*0.05>*p*>0.01; **0.01>*p*>0.001;

****p*<0.001

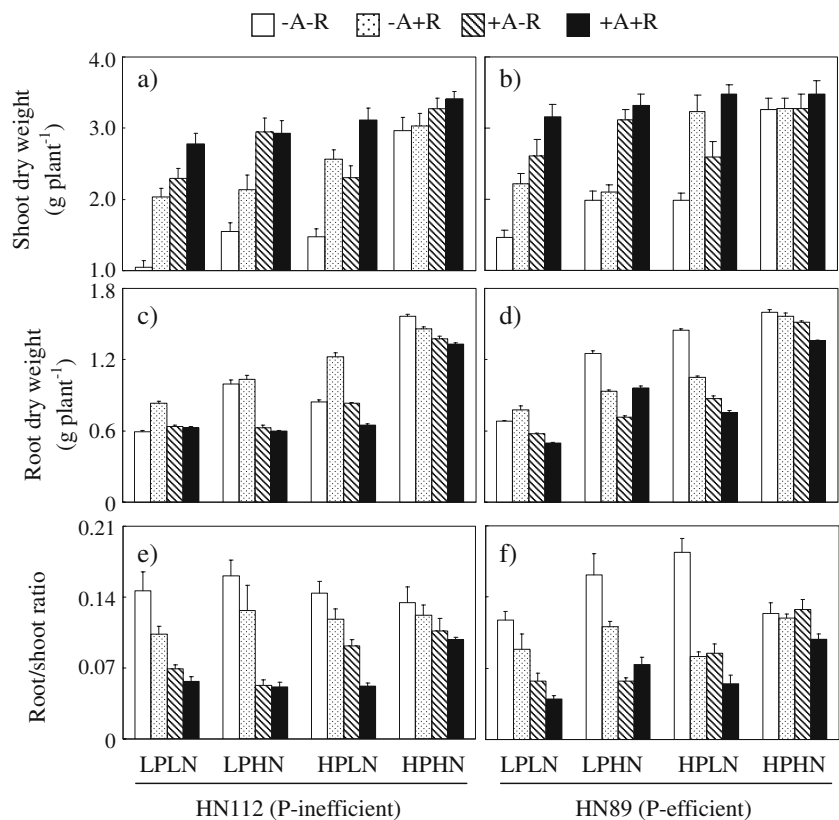
Soil pot experiment

Plant growth performance

Soybean plant growth was significantly affected by AM fungi and rhizobia inoculation, and N and P availability as indicated by dry weight of shoots and roots, and root/shoot

ratio (Fig. 1). At low N and/or low P levels, inoculation with rhizobia and/or AM fungi dramatically increased soybean shoot dry weight, and decreased root/shoot ratio (Fig. 1a, b, e, and f). Plants co-inoculated with rhizobia and AM fungi had higher shoot dry weight and lower root/shoot ratio, particularly under low P and low N conditions for HN112. The shoot dry weight of HN112 was increased

Fig. 1 Plant growth performance as affected by AM fungi and rhizobia inoculation under different N and P conditions. **a** and **b** Shoot dry weight; **c** and **d** root dry weight; **e** and **f** root/shoot ratio. *HPHN* high P and high N treatment, *HPLN* high P and low N treatment, *LPHN* low P and high N treatment, *LPLN* low P and low N treatment; +*A+R* co-inoculation with AM fungi and rhizobia, +*A-R* inoculation with AM fungi, -*A+R* inoculation with rhizobia, -*A-R* no-inoculation. Each bar represents the mean of four replicates with standard error



166%, but only 115% for HN89 by co-inoculation compared with the uninoculated treatment in the LPLN treatment. Furthermore, in the LPHN treatment, inoculation with rhizobia alone had much less influence on shoot dry weight and root/shoot ratio than inoculation with AM fungi alone or co-inoculation with rhizobia.

Effects of AM fungi and rhizobia inoculation and N and P availability on root dry weight were more complicated (Fig. 1c, d). AM inoculation generally inhibited root growth as indicated by less root dry weight compared to inoculation with rhizobia alone or without inoculation. This inhibition effect was more significant in the P-efficient genotype HN89. For example, under HPLN conditions, co-inoculation with rhizobia and AM fungi decreased root dry weight of HN89 and HN112 by 92.50% and 29.50% compared to the no-inoculation treatment, respectively. Furthermore, effects of rhizobia inoculation on root dry weight differed between the soybean genotypes. For HN112, rhizobia inoculation alone significantly stimulated root growth under low N. The root dry weight of HN112 was increased 40.43% and 45.38% by inoculation with rhizobia alone compared to the no-inoculation treatment under LPLN and HPLN conditions, respectively. In contrast, for HN89, inoculation with rhizobia inhibited root growth. The root dry weight of HN89 decreased 25.54% and 27.61% under LPHN and HPLN conditions, respectively, when inoculated with rhizobia alone compared to the no-inoculation treatment.

Plant N and P status

Plant N and P status, as indicated by N and P content, was significantly affected by AM fungi and rhizobia inoculation, and N and P availability (Fig. 2). Under low N

conditions, inoculation with rhizobia and/or AM fungi dramatically increased plant N content for both genotypes compared to no-inoculation treatments (Fig. 2a, b). Under low P conditions, with sufficient N, only AM fungal inoculation led to increased N content. Co-inoculation with both rhizobia and AM fungi resulted in a 136.70% and 129.33% increase of plant N content for HN112 and HN89 compared to no-inoculation treatments, respectively.

Inoculation with rhizobia and/or AM fungi significantly increased plant P content of HN112 at low N and/or low P levels. Plant P content of HN89 only increased at low P when inoculated with solely AMF (Fig. 2c, d). Still, inoculation with AM fungi increased plant P content of HN112 more than HN89 at low P. For instance, at LPLN, inoculation with AM alone or co-inoculation increased plant P content of HN112 178.97% or 214.90%, respectively, but only 51.44% or 102.80% for HN89. This indicated that the P status of HN112 was more responsive to rhizobia and/or AM fungi inoculation.

Root traits

Inoculation with AM fungi significantly inhibited root growth as indicated by less total root length, smaller root surface area, and less root volume compared to the treatment with no inoculation, especially in LNLP conditions (Fig. 3). For example, inoculation with AMF alone decreased root length 28.55% and 19.83% for HN112 and HN89 in the LNLP treatment level, respectively. There were synergistic effects of co-inoculation with rhizobia and AM fungi on root growth, especially for HN112 at the LNLP level. Co-inoculation, compared to no-inoculation treatments, reduced root length, surface area, and volume of HN112 and HN89 39.95% and 24.53%, 41.03% and 21.83%, 43.14% and

Fig. 2 Plant N and P content as affected by AM fungi and rhizobia inoculation under different N and P conditions. **a** and **b** plant N content; **c** and **d** plant P content; *HPHN* high P and high N treatment, *HPLN* high P and low N treatment, *LPHN* low P and high N treatment, *LPLN* low P and low N treatment, *+A+R* co-inoculation with AM fungi and rhizobia, *+A-R* inoculation with AM fungi, *-A+R* inoculation with rhizobia, *-A-R* no-inoculation. Each bar represents the mean of four replicates with standard error

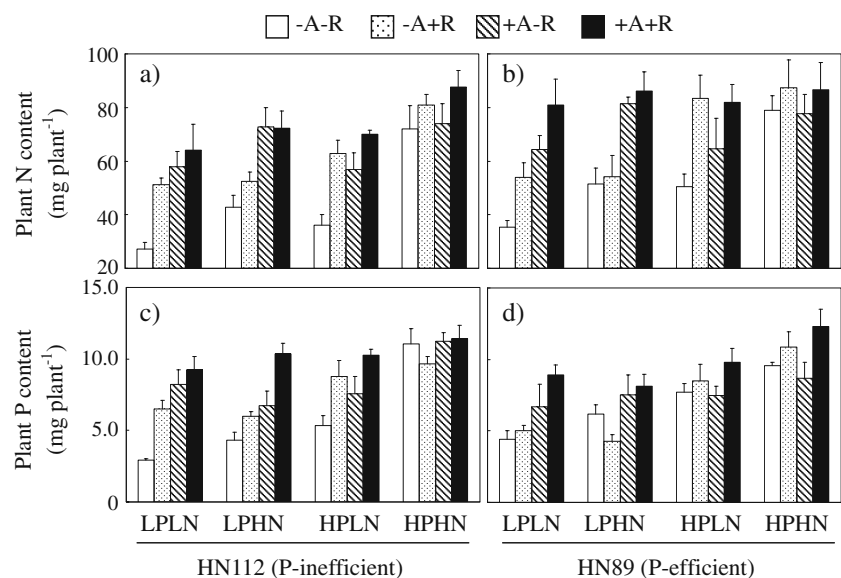
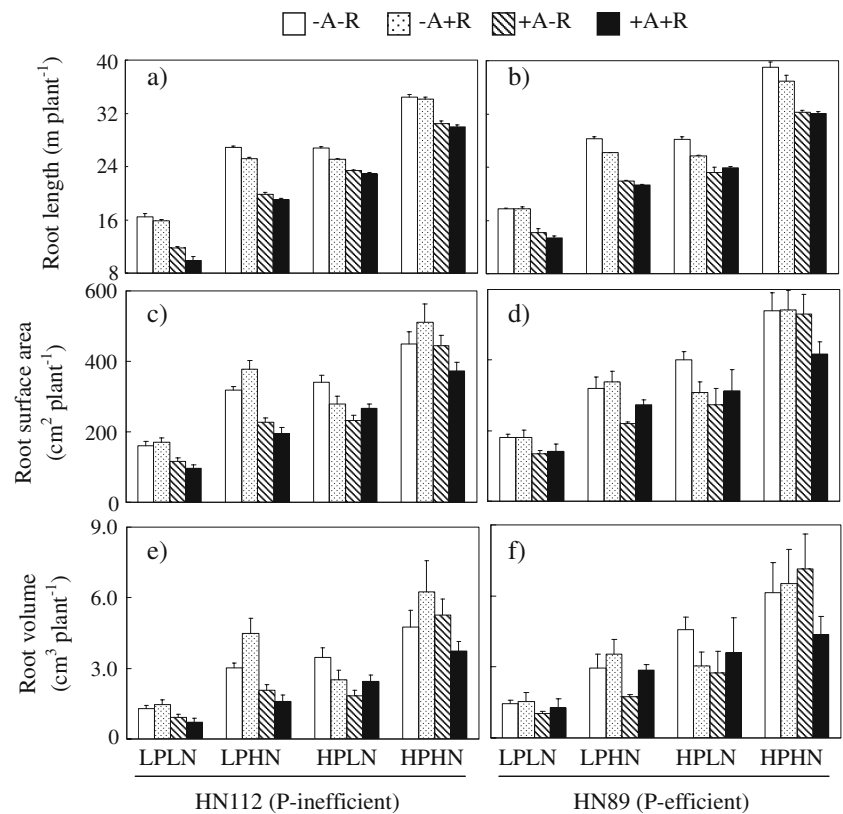


Fig. 3 Root morphological traits as affected by AM fungi and rhizobia inoculation under different N and P conditions. **a** and **b** root length; **c** and **d** root surface area; **e** and **f** root volume. *HPHN* high P and high N treatment, *HPLN* high P and low N treatment, *LPHN* low P and high N treatment, *LPLN* low P and low N treatment, *+A+R* co-inoculation with AM fungi and rhizobia, *+A-R* inoculation with AM fungi, *-A+R* inoculation with rhizobia, *-A-R* no-inoculation. Each bar represents the mean of four replicates with standard error



12.07%, respectively. Interestingly, inoculation with rhizobia alone did not change root length, but slightly increased root surface area and volume of HN112 at LPHN level (Fig. 3c, e).

AMF colonization and nodulation

AMF colonization was enhanced by inoculation with AM fungi alone or co-inoculation with rhizobia, especially under low P conditions for HN112 (Fig. 4a, b). Under low P conditions, co-inoculation with rhizobia and AM fungi had synergistic effects on AMF colonization as indicated by higher AMF colonization with co-inoculation than inoculation with AM fungi alone. At LPLN and LPHN levels, AMF colonization in co-inoculation compared with no-inoculation for HN112 was increased 7.9 and 7.3 times, respectively, while for BX10 they increased 4.3 and 5.8 times, respectively. This also showed that HN112 had higher AMF colonization.

Soybean nodulation was promoted by inoculation with rhizobia as indicated by nodule number and fresh weight (Fig. 4c–f). Co-inoculation with rhizobia and AM fungi also had synergistic effects on soybean nodulation, especially under low P and low N conditions for HN112. In the LPLN treatment, co-inoculation increased nodule number and fresh weight of HN112 13.3 and 253.3 times, respectively, and for HN89 they were increased 16.6 and 56.9 times, respectively. Furthermore, nodule number and

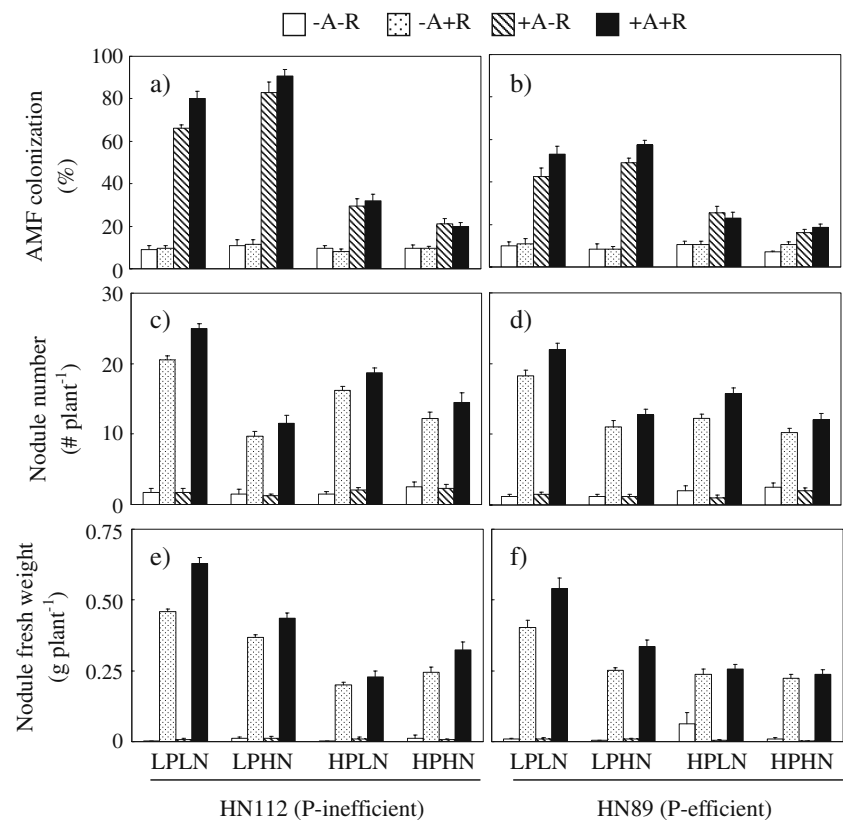
fresh weight were always highest under simultaneous P and N stress in both soybean genotypes whether inoculated with rhizobia alone or co-inoculated with AM fungi, indicating that soybean nodulation was more sensitive to N and P availability than mycorrhiza development.

Discussion

AM associations have been shown to induce modifications in root architecture and morphogenesis in herbaceous plants as well as in trees, but with inconsistent results for different plants and/or fungal species (Oláh et al. 2005; Hodge et al. 2009; Yao et al. 2009). Some studies found that AMF colonization enhanced root branching as well as root length, such as in leek (Berta et al. 1990), *Vitis vinifera* (Schellenbaum et al. 1991) and carob (Cruz et al. 2004). In contrast, Yao et al. (2009) reported that AM fungi significantly reduced the total root length, the root surface area, and the root volume in trifoliate orange. We also found that AMF colonization significantly reduced the total root length, the root surface area, and the root volume in soybean (Fig. 3). This indicated that the effects of AM association on root architecture may be plant and/or fungal species dependent and need to be further studied.

Nodules are well known as useful symbioses for biological N fixation in legumes. But there were very few

Fig. 4 AMF colonization and nodulation as affected by AM fungi and rhizobia inoculation under different N and P conditions. **a** and **b** AMF colonization; **c** and **d** nodule number; **e** and **f** nodule fresh weight. *HPHN* high P and high N treatment, *HPLN* high P and low N treatment, *LPHN* low P and high N treatment, *LPLN* low P and low N treatment, *+A+R* co-inoculation with AM fungi and rhizobia, *+A-R* inoculation with AM fungi, *-A+R* inoculation with rhizobia, *-A-R* no-inoculation. Each bar represents the mean of four replicates with standard error



studies on the relationship between nodulation and plant root architecture. In nature, legumes such as soybean can form symbiotic associations with both rhizobia and AM fungi (Lisette et al. 2003). Therefore, tripartite symbiotic associations with rhizobia and AM fungi should be very important for soybean nutrient acquisition, and the effects of inoculation with rhizobia and AM fungi on soybean roots need to be further researched.

From the field experiment, we found that the responses of soybean AMF colonization and nodulation to P availability under natural conditions were dependent upon root architecture (Table 2). The deep root genotype, HN112, had greater AMF colonization at low P, but better nodulation at the high P level. Even though mycorrhizae can greatly enhance the acquisition of mineral nutrients (Marshchner and Dell 1994), they also cost a lot of carbon from the host plants (Fitter 2006). Therefore, if the host plants have the ability to take up enough nutrients, such as P, they might not form many mycorrhizae due to the balance of the C and P budgets (Smith et al. 2009). It was reported that legumes with shallower root architectures had better spatial configuration in the cultivated soil surface layer with higher P levels and thus had higher P acquisition efficiency and yield (Liao et al. 2004; Zhao et al. 2004). In the present study, the shallow root genotype, HN89, always has a wider root system as well as higher P efficiency as indicated by plant biomass, and N and P content (Table 2).

For this genotype, lower AMF colonization exists in both field and soil pot experiments under low P conditions (Table 2, Fig. 4b). On the other hand, the deep root genotype, HN112, had higher AMF colonization but lower P efficiency (Table 2, Fig. 4a), indicating a complementary relationship between root architecture and AMF colonization on soybean P efficiency.

From the soil pot experiment, co-inoculation with rhizobia and AM fungi significantly increased soybean growth as indicated by increased shoot dry weight, and plant N and P content in the low P and/or low N nutrient treatment levels, but not in the high P and high N nutrient treatment levels (Fig. 1). These results indicated that there was a synergistic effect between rhizobia and AM fungi on soybean growth in this study and that this effect is dependent upon nutrient status. It is believed that mycorrhizae especially benefit plants grown in soils where P is likely to limit plant growth by increasing the soil volume explored by AM hyphae relative to that of root hairs of non-AM plants (Bolan 1991; Jakobsen 1995). This may explain the synergistic effects of co-inoculation with AM fungi and rhizobia which have been reported for different crops, and why such effects are more pronounced in soils deficient in both N and P as we found in the present study. The general consensus is that AM fungi improve P nutrition of legumes, which in turn enhances plant growth and N fixation (Barea and Azcon-Aguilar 1983; Cluett and

Boucher 1983). Lisette et al. (2003) reported that co-inoculation with rhizobia and compatible AM fungi could dramatically enhance pea growth, and N and P uptake. Therefore, the AM fungi we used for the present study are compatible with our rhizobial strain and soybean genotype, which might have potential for agricultural application.

Besides the general synergistic effect of co-inoculation with rhizobia and AM fungi on soybean growth, our results also showed that this effect was related to root architecture. The two tested soybean genotypes, HN112 and HN89 have been shown to be contrasting in root architecture (Table 2, Zhao et al. 2004). The deep root genotype, HN112, has greater responses to co-inoculation as indicated by much greater increases in shoot dry weight and P content compared to HN89 under low P and/or low N conditions (Figs. 1a,b and 2c,d). Interestingly, there are less genotypic differences of plant N content in response to co-inoculation (Fig. 2a,b), indicating this synergistic effect is mainly caused by the improvement in P, rather than N, nutrient status. This is also demonstrated by the results from the field. HN112 had higher AMF colonization at low P but greater nodulation with high P supply (Table 2), indicating that P nutrient status is the main limiting factor in soybean nodulation in nature, particularly for the deep root genotype. It is also thought that the plant-*Rhizobium* system benefits from the presence of AM fungi because the mycorrhizae ameliorate not only P deficiency but also any other nutrient deficiencies that might be limiting to *Rhizobium* (Pacovsky 1986; Smith 2002). Increased mineral nutrient levels in the plants would not only benefit *Rhizobium* directly, but would also lead to increased photosynthesis, making a greater proportion of photosynthates available to the *Rhizobium* nodules (Harris et al. 1985; Mortimer et al. 2008). Therefore, the mechanisms by which the deep root genotype benefits more from co-inoculation with rhizobia and AM fungi might be the improved P status caused by AMF colonization and/or enhanced nodulation due to improved P and other nutrient statuses.

In conclusion, our results have verified the important effects of co-inoculation with rhizobia and AM fungi on soybean growth and the synergistic relationship between rhizobia and AM fungi. It was also found that P and N status could influence the effectiveness of inoculation, which was also related to genotypic difference in root architecture. The deep root soybean genotype had greater plastic responses of both AMF colonization and nodulation to N and P availability. These results could help us gain a better insight into the effects of rhizobia and AM fungi on soybean growth, providing a theoretical basis for practical application of co-inoculation in field-grown soybean.

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