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Original Article

Growth and Photosynthetic Responses of Litchi Seedlings to Arbuscular Mycorrhizal Fungal Inoculation: Differences between Two Genotypes

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Abstract

Arbuscular mycorrhizal (AM) fungi are beneficial symbiotic soil microorganisms and AM technology can find its potential application in the nursery of horticultural industry. When AM fungi have been successfully applied to many wood fruit tree species, little information is available in litchi (*Litchi chinensis* Sonn.). In this study, the seedlings of two litchi genotypes ('Baila' and 'Heiye') were inoculated with two AM fungal species (*Rhizophagus irregularis* and *Gigaspora margarita*) in the nursery conditions, and the growth and photosynthetic responses of seedlings to AM fungal inoculation were investigated. Results indicated that AM fungi significantly promoted the plant growth of 'Heiye' seedlings in terms of biomass, plant height, stem diameter and leaf number, while they slightly decreased these parameters of 'Baila'. The inoculation effect can be explained by the changes in photosynthetic characteristics induced by AM fungi, because AM fungi increased A_{max}, A_{qe}, LSP and decreased LCP of 'Heiye' but did not affected those of 'Baila'. P_n was not affected by AM fungi, however, regression analysis indicated a weaker relationship between biomass and P_n than those between biomass and A_{max}, LSP or LCP. Our results strongly suggest that AM fungi can differentially affect the seedling growth of litchi genotypes mainly via their effects on photosynthetic characteristics, and that precautions should be taken to select appropriate genotypes as rootstock if AM technology is applied in litchi nursery.

Keywords: *Gigaspora margarita*; growth enhancement; *Litchi chinensis*; nursery; photosynthesis; *Rhizophagus irregularis*

Introduction

Litchi (*Litchi chinensis* Sonn.) is subtropical fruit tree native to the area between southern China, northern Viet Nam and Myanmar (Menzel, 2002). China is the leading litchi-producing country in the world with 950 thousand metric tons of production in 2002 (Jiang *et al*., 2012), however, most litchi orchards in China are located in hilly and marginal soils. These soils are typical of poor soil structure, low fertility and low pH (Wang *et al*., 2012), indicating the necessity of soil improvement and appropriate fertilization scheme in these orchards. In addition to those conventional strategies, however, some novel biotechnologies, such as arbuscular mycorrhizal (AM) technology, can also be relied on, especially when orchard sustainability is considered (Azcón-Aguilar and Barea, 1997). AM fungi (phylum Glomeromycota) are a kind of soil fungi, ubiquitous in all terrestrial ecosystems (Rillig, 2004). They establish symbiotic relationship with plant roots and, consequently, increase their host resistance to diverse biotic and/or abiotic stresses (Zhu and Yao, 2004; Zhu *et al*., 2007; Sensoy *et al*., 2013; Yao *et al*., 2014; Zou *et al*., 2014). Due to their multiple functions, Azcón-Aguilar and Barea (1997) regarded these symbiotic fungi as biofertilizers and bioprotectors in horticulture. In this context, it is clearly plausible to apply AM technology to the production of litchi in southern China.

The colonization of litchi plant roots by indigenous AM fungi in orchards has been frequently reported (Singh and Prasad, 2006; Sharma *et al*., 2009), and the diversity of these indigenous AM fungi has also been investigated (Mridha and Dhar, 2007; Sharma *et al*., 2009). It was found that root colonization of litchi plants and AM fungal spore density in the rhizosphere were as high as 65.42% and 2010 per 100 g dry soil in field conditions (Singh and Prasad, 2006). The Shannon's diversity index of indigenous AM fungi in litchi

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orchard soils was nearly 2.0, with *Glomus* species accounting for 83% (Mridha and Dhar, 2007). For the application of AM fungi to litchi production, the inoculation effect has been evaluated with either indigenous or exogenous AM fungal species. In an air-layer propagation system, Janos *et al*. (2001) found inoculation with the indigenous fungal species increased the aboveground biomass of litchi plants by 39% although the root colonization was only 7.4%. Similarly, Sharma *et al*. (2009) inoculated the air-layers with four indigenous AM fungal species in combination with two *Azotobacter chroococcum* strains, and indicated that both AM fungi and *Azotobacter* promoted the total length of adventitious roots with a positive interactive effect. In an experiment using vermicompost as substrate, indigenous AM fungi promoted the plant height, leaf number and shoot length of air-layers by 132%, 750% and 68% (Sagar and Roy, 2013). While these results demonstrate the potential application of AM fungi in the air-layer propagation system, grafting propagation system is widely recognized in China because seedlings in this system are more tolerant to stresses with better developing roots. Therefore, the evaluation of AM fungal inoculation effect on the growth of rootstock seedlings is of practical importance. Yao *et al*. (2005) reported that *Glomus intraradices* and *Gigaspora margarita* increased the biomass of rootstock seedlings by 13.5%-30.1% regardless of low root colonization (<18.8%). The enhanced P uptake and phytohormones (IAA and iPAs) seemed to account for the changes in plant growth. In general, however, information of AM fungal effect on rootstock seedlings is very scarce.

Photosynthesis is the basis of carbohydrate accumulation in plants, and the improved photosynthesis together with enhanced nutrient uptake by AM fungi was demonstrated to contribute much to the increase in biomass of many plants (Birhane *et al*., 2012; Zhu *et al*., 2012). In our study, we focused on the effects of AM fungal inoculation on the photosynthetic characteristics, other than the nutrient status, of litchi rootstock seedlings, and meanwhile, the difference of two rootstock genotypes was also under investigation.

Materials and Methods

Plant material, fungal species and substrate

To investigate the AM fungal inoculation effect in nursery, all the steps in our study followed the procedure in litchi nursery in southern China, except that the sterilization and inoculation.

Two genotypes of litchi (*Litchi chinensis* Sonn.), 'Baila' and 'Heiye', were taken as rootstocks tested in our study. They were routinely used as rootstocks in litchi nursery due to the high quality seeds and vigorous seedlings. AM fungal species for inoculation were *Rhizophagus irregularis* (formerly known as *Glomus intraradices*) and *Gigaspora margarita*, which were used to colonized litchi seedlings in previous experiment (Yao *et al*., 2005). AM fungal inoculum was the mixture of soil, sands and colonized root fragments, propagated with clover and sorghum as host plants. Substrate for litchi propagation was the mixture of orchard soils, fermented chicken manure and peat (2:1:1 in volume), which followed the routine formula for litchi nursery. Orchard soils were from the litchi orchard of College of Horticulture, South China Agricultural University, while fermented chicken manure and peat were from the market. To exclude the indigenous AM fungal community, orchard soils and peat were sterilized using Co^{60} irradiation with dose of 20.0 kGy. The chemical properties of orchard soils were as follows: pH 5.25, soil organic matter content 58.95 $g{\cdot}kg^{-1}$, total N content 2.29 $g{\cdot}kg^{-1}$, total P content 1.06 $g{\cdot}kg^{-1}$, total K content 8.63 $g{\cdot}kg^{-1}$, available N content 394.50 $mg{\cdot}kg^{-1}$, available P content 40.13 $mg{\cdot}kg^{-1}$, available K content 179.65 $mg{\cdot}kg^{-1}$.

Experimental design and seedling growth

Litchi seeds were germinated in autoclaved vermiculite and let grow until the plant height reached 10-15 cm. Then seedlings of similar vigor were transplanted to black plastic nursery bag of 7.85 liters (1 seedling per bag), with the substrate inoculated with *R. irregularis*, *G. margarita* or not inoculated (control). There were 3 inoculation treatments and 50 replicates for each treatment, thus producing 150 nursery bags in our study. Seedlings were placed in a glasshouse and grew for 12 months before harvest. The glasshouse was cooled at high temperature in summer but not heated at low temperature in winter, with temperature range of 18 °C-30 °C and natural light intensity. During the growth, seedlings were watered regularly and fertilized once at the sixth month with 200 mg N and 100 mg K per kg substrate.

Measurement of parameters

Before harvest, 10 seedlings were randomly taken out of 50 seedlings in each treatment for measurement of parameters. Plant height, leaf number and stem diameter were recorded. SPAD of the fully expanded leaves was measured using a chlorophyll meter (Minolta SPAD-502). After these non-destructive measurements, the photosynthesis of the seedlings was characterized using a portable photosynthesis system (Li-6400, Li-COR Biosciences Inc, Lincoln, USA). Then seedlings were carefully harvested, and roots were cut off and cleared of soils with tap water. Shoot fresh weight and root fresh weight were recorded. Accordingly, mycorrhizal dependency was calculated as 100% × (biomass of inoculated seedling – biomass of un-inoculated seedling) / biomass of inoculated (Yao *et al*., 2009). Chlorophyll was extracted and quantified according to Asmar *et al*. (2013). About 1 g fine roots were cut off for the quantification of AM fungal colonization. Briefly, root fragments of 1-2 cm length were stained according to Koske and Gemma (1989) and then observed under microscope at ×200 magnifications. Root colonization, percentage of arbuscules and percentage of vesicles were quantified according to McGonigle *et al*. (1990).

Photosynthetic parameters were measured in the morning at 9:00-11:00. For the photosynthetic rate, stomatal conductance and transpiration rate, determination was performed at 25 °C with 60% relative humidity, 350 $\mu mol{\cdot}mol^{-1}$ CO_2 concentration and 1000 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ light intensity. For plotting the light response curve, light intensity was set at 20, 50, 100, 300, 500, 800, 1000, 1200, 1400, 1600, 1800, 2000 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ with other conditions the same as above.

Statistical analyses

All data were the average of ten replicates. Two-way analysis of variance (ANOVA) and multiple range test (Tukey's) were performed using SPSS 17.0 software (SPSS Inc., Chicago, USA). Light response curves were fitted by nonlinear regression with the Mitscherlich model equation using SigmaPlot 12.5 software (SSPS Inc., Chicago, USA) as follows: $A = A_{max} [1 - e^{-Aqe(PPFD-LCP)}]$, where A is net photosynthesis (P_n), A_{max} refers to the asymptote of photosynthesis, A_{qe} represents the apparent quantum yield or initial slope of the curve, PPFD is the incident photosynthetic photon flux density, and the LCP refers to the light compensation point that corresponds to the x-intercept (where photosynthetic uptake and respiratory CO_2 release are in equilibrium). The calculation of A_{max}, A_{qe}, LCP, light saturation point (LSP) and dark respiration rate (R_d) was according to Aleric and Kirkman (2005). Relationships between shoot biomass and P_n, A_{max}, LCP or LSP were analyzed with linear regression using SigmaPlot 12.5 software.

Results

AM fungal colonization in the roots of rootstock seedlings

Data in Table 1 showed that both *R. irregularis* and *G. margarita* colonized the roots well, with colonization rate varying between 54.4% and 74.8%. When the percentages of arbuscules in roots were 10.3%-18.3%, the percentages of vesicles in roots colonized by *R. irregularis* were only 2.2%-5.8% with no vesicles present in those colonized by *G. margarita*. It is clear that all these three parameters of 'Heiye' were significantly higher than those of 'Baila' (Table 1). There is no significant interaction between genotype and inoculation.

AM fungal inoculation effect on the growth of rootstock seedlings

It is clear that shoot biomass and plant height of 'Baila' were significantly higher than those of 'Heiye' and inoculation significantly affected shoot biomass, stem diameter and leaf number (Table 2). Data indicated that AM fungi promoted the seedling growth of 'Heiye' but not of 'Baila' (Table 2). In detail, both *R. irregularis* and *G. margarita* significantly increased the shoot biomass of 'Heiye' although the root biomass was not affected. In contrast, *R. irregularis* significantly decreased both shoot biomass and root biomass of 'Baila', and *G. margarita* showed no effect. For plant height and stem diameter, AM fungal inoculation effect exhibited similar patterns to that for the biomass except that *G. margarita* showed no inoculation effect on 'Heiye' (Table 2). For leaf number, both *R. irregularis* and *G. margarita* did not affect 'Baila' but significantly increased that of 'Heiye' (Table 2).

In general, AM fungal inoculation promoted the seedling growth of 'Heiye', but did not affect and even decreased the seedling growth of 'Baila'. Accordingly, the mycorrhizal dependencies of 'Heiye' were 17.4% (*R. irregularis*) and 12.1% (*G. margarita*), while those of 'Baila' were -31.7% (*R. irregularis*) and 3.5% (*G. margarita*) (Table 2). These data demonstrate the great difference in the growth responses of 'Baila' and 'Heiye' seedlings to AM fungal inoculation.

AM fungal inoculation effect on the photosynthetic characteristics of rootstock seedlings

Data in Table 3 indicate that chlorophyll contents in the leaves of 'Heiye' were much higher than those of 'Baila', and in contrast, the transpiration rate of 'Heiye' was lower than that of 'Baila'. AM fungal inoculation significantly increased SPAD values but decreased the stoma conductivity and transpiration rate.

For 'Baila', both *R. irregularis* and *G. margarita* did not significantly affect SPAD value, chlorophyll contents, net photosynthesis, stoma conductivity and transpiration rate (Table 3). For 'Heiye', chlorophyll contents were not affected by AM fungal, while SPAD value was significantly increased. When *R. inregularis* slightly increased the net photosynthesis, stoma conductivity and transpiration rate, *G. margarita* slightly decreased them (Table 3). No significant difference between non-inoculated and inoculated seedlings was observed, while significant difference between seedlings inoculated with two AM fungal species was observed (Table 3). Light response curves of seedlings were fitted by nonlinear regression (Fig. 1).

Table 1. Root colonization, percentage of arbuscules and percentage of vesicles by AM fungi in the seedling roots of 'Baila' and 'Heiye'

Inoculation treatments	Root colonization (%)	Percentage of arbuscules (%)	Percentage of vesicles (%)
'Baila'			
Non-inoculation	0±0 b	0±0 b	0±0 b
Rhizophagus irregularis	54.4±4.8 a	10.3±1.3 a	2.2±0.6 a
Gigaspora margarita	57.8±4.6 a	13.3±2.0 a	NA
'Heiye'			
Non-inoculation	0±0 c	0±0 b	0±0 b
Rhizophagus irregularis	74.6±4.5 a	16.9±1.8 a	5.8±1.6 a
Gigaspora margarita	57.8±4.7 b	18.3±1.9 a	NA
Two-way ANOVA			
Cultivar	*	*	*
Inoculation	***	***	**
Cultivar× Inoculation	ns	ns	ns

Note: "NA" indicates "not applicable". "*", "**", "***" and "ns" indicate significant difference at $p<0.05$, $p<0.01$, $p<0.001$ and not significant difference.

Table 2. Influence of AM fungal inoculation on the biomass, plant height, stem diameter and leaf number of 'Baila' and 'Heiye' seedlings and their mycorrhizal dependencies

Inoculation treatments	Shoot FW (g)	Root FW (g)	Plant height (cm)	Stem diameter (mm)	Leaf number	Mycorrhizal dependency (%)
'Baila'						
Non-inoculation	34.3±2.0 a	7.3±0.9 a	51.4±1.8 a	6.1±0.1 ab	22.1±1.4 a	—
Rhizophagus irregularis	26.4±2.2 b	5.3±0.4 b	47.3±1.1 b	5.8±0.1b	20.4±1.1 a	-31.2
Gigaspora margarita	36.2±3.2 a	6.9±0.9 a	50.5±1.0 ab	6.5±0.1 a	21.5±1.8 a	3.5
'Heiye'						
Non-inoculation	21.7±2.5 b	5.9±0.5 a	43.1±1.8 b	5.8±0.2 b	21.1±1.6 b	—
Rhizophagus irregularis	27.7±2.0 a	5.7±0.6 a	48.5±1.7 a	6.3±0.2 a	25.9±1.8 a	17.4
Gigaspora margarita	26.1±0.6 a	5.3±0.6 a	44.8±1.1 ab	5.8±0.1 b	24.5±1.5 a	12.1
Two-way ANOVA						
Cultivar	*	ns	*	ns	ns	
Inoculation	*	ns	ns	*	*	
Cultivar× Inoculation	ns	ns	*	ns	*	

Note: "*", "**", "***" and "ns" indicate significant difference at $p<0.05$, $p<0.01$, $p<0.001$ and not significant difference.

Table 3. Influence of AM fungal inoculation on the photosynthetic characteristic of 'Baila' and 'Heiye' seedlings

Inoculation treatments	SPAD value	Chla content ($mg.g^{-1}$)	Chlb content ($mg.g^{-1}$)	Pn ($\mu mol\ CO_2.m^{-2}.s^{-1}$)	Cs ($mmol\ H_2O.m^{-2}.s^{-1}$)	Tr ($mmol\ H_2O\ m^{-2}.s^{-1}$)
'Baila'						
Non-inoculation	47.1±0.7 a	0.09±0.02 a	0.20±0.05 a	4.18±0.63 a	8.0±1.3 a	0.43±0.07 a
Rhizophagus irregularis	47.4±1.1 a	0.08±0.01 a	0.15±0.02 a	4.06±0.47 a	7.2±1.2 a	0.40±0.06 a
Gigaspora margarita	48.0±0.8 a	0.10±0.01 a	0.19±0.02 a	3.82±0.35 a	6.7±1.0 a	0.37±0.05 a
'Heiye'						
Non-inoculation	43.2±1.0 b	0.14±0.01 a	0.35±0.02 a	3.87±0.31 ab	7.1±1.0 ab	0.38±0.03 ab
Rhizophagus irregularis	47.3±09 a	0.16±0.01 a	0.40±0.02 a	4.58±0.48 a	8.2±0.7 a	0.44±0.06 a
Gigaspora margarita	46.8±1.0 a	0.16±0.01 a	0.39±0.02 a	2.88±0.31 b	5.4±0.8 b	0.29±0.03 b
Two-way ANOVA						
Cultivar	ns	*	*	ns	ns	*
Inoculation	*	ns	ns	ns	*	*
Cultivar× Inoculation	ns	ns	ns	ns	ns	ns

Note: "*", "**", "***" and "ns" indicate significant difference at $p<0.05$, $p<0.01$, $p<0.001$ and not significant difference.

Table 4. Influence of AM fungal inoculation on the light response curves of 'Baila' and 'Heiye' seedlings

Inoculation treatments	A_{max} ($\mu mol\ CO_2.m^{-2}.s^{-1}$)	A_{qe}	LCP ($\mu mol.m^{-2}.s^{-1}$)	LSP ($\mu mol.m^{-2}.s^{-1}$)	R_d ($\mu mol\ CO_2.m^{-2}.s^{-1}$)
'Baila'					
Non-inoculation	6.65±0.42 a	0.05±0.01 a	7.95±0.84 ab	611.26±48.63 a	-0.40±0.04 a
Rhizophagus irregularis	7.67±0.30 a	0.10±0.05 a	6.75±0.85 b	678.26±52.10 a	-0.33±0.04 a
Gigaspora margarita	7.00±0.34 a	0.10±0.05 a	9.68±1.11 a	731.76±57.84 a	-0.46±0.07 a
'Heiye'					
Non-inoculation	3.99±0.14 b	0.02±0.00 b	41.96±17.98 a	429.67±41.86 b	-0.75±0.05 a
Rhizophagus irregularis	6.40±0.22 a	0.04±0.02 ab	7.94±3.97 b	804.30±69.10 a	-0.25±0.18 a
Gigaspora margarita	6.38±0.15 a	0.07±0.04 a	11.92±3.97 b	645.63±61.74 ab	-0.82±0.58 a
Two-way ANOVA					
Cultivar	**	**	*	ns	ns
Inoculation	*	*	**	*	ns
Cultivar× Inoculation	ns	ns	*	*	ns

Note: "*", "**", "***" and "ns" indicate significant difference at $p<0.05$, $p<0.01$, $p<0.001$ and not significant difference.

According to these curves, A_{max} and A_{qe} of 'Baila' were significantly higher than those of 'Heiye' while LCP of 'Baila' was lower than that of 'Heiye'. AM fungal inoculation significantly affected the A_{max}, A_{qe}, LCP, LSP, but not the R_d (Table 4). However, 'Baila' and 'Heiye' exhibited different response to AM fungal inoculation. The A_{max}, A_{qe} and LSP of 'Baila' seedlings were not affected by AM fungal inoculation but those of 'Heiye' seedlings were significantly increased, meanwhile, LCP of 'Heiye' was significantly decreased by AM fungal inoculation (Table 4). The R_d of both genotypes was not affected by AM fungal inoculation. These results indicate the much different response of the photosynthetic characteristics of 'Baila' and 'Heiye' seedlings to AM fungal inoculation.

Relationship between plant biomass and photosynthetic parameters

To evaluate the relative importance of photosynthetic parameters in the seedling growth, linear regression was performed between shoot biomass and P_n, A_{max}, LCP or LSP. It is clear that seedling growth was positively related to P_n, A_{max} and LSP, but negatively related to LCP (Fig. 2). However, the R values of A_{max}, LCP and LSP were around or above 0.5000 while that of P_n was as low as 0.1790. This indicates the more suitability of photosynthetic parameters derived from light response curve to predict the plant growth than the photosynthetic rate measured at a certain time point.

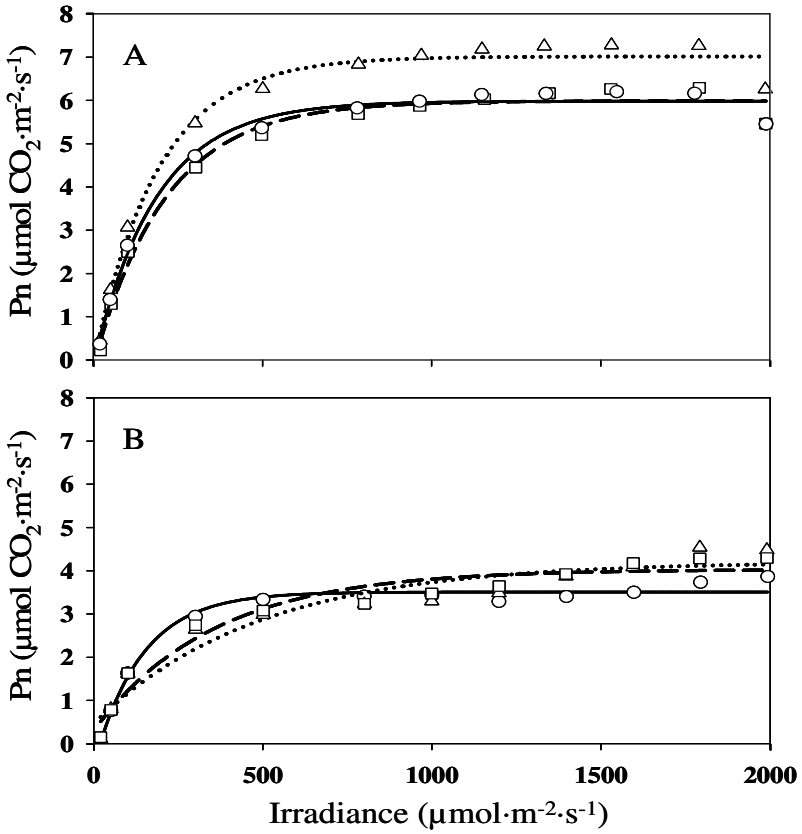

Fig. 1. Light response curves of photosynthesis as a function of irradiance for two genotypes of litchi, 'Baila' (A) and 'Heiye' (B). Plants were inoculated with *Gigaspora margarita* (dotted line with triangle symbols), *Rhizophagus irregularis* (dashed line with square symbols) or non-inoculated (solid line with circle symbols). Each data point (symbol) represents means of 10 replicates. Light curves were fitted by nonlinear regression using the Mitscherlich model equation.

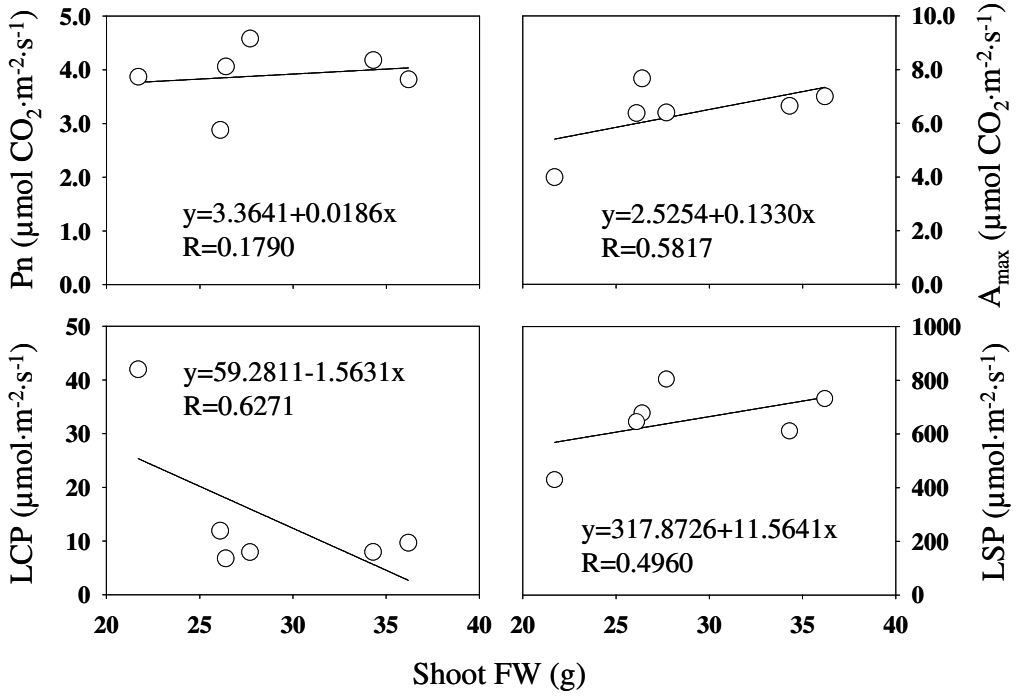

Fig. 2. Relationships between shoot biomass and net photosynthesis (P_n), asymptote of photosynthesis (A_{max}), light compensation point (LCP) or light saturation point (LSP). Each point represents the average of ten replicates. Linear regression was performed using SigmaPlot 12.5 software.

Discussion

Growth response of litchi seedlings to AM fungi

The growth responses of plants to AM fungal colonization vary greatly. It seems that woody plants are less sensitive than non-woody plants. In a meta-analysis, Hoeksema *et al*. (2010) categorized plants into several functional groups and listed them in the following order in terms of growth response to AM fungi: C4 grasses > non-N-fixing forbs > non-N-fixing woody > C3 grasses > N-fixing forbs > N-fixing woody. In our study, mycorrhizal dependency of litchi rootstock seedlings is below 20%. In the previous experiment, the mycorrhizal dependencies varied from 13.5% to 30.1% (Yao *et al*., 2005). In the air-layer system, mycorrhizal dependency was about 40% (Janos *et al*., 2001). Collectively, these data suggest that litchi, as woody plant, can benefit from AM fungal inoculation to a certain extent in terms of plant growth. Habte and Manjunath (1991) have proposed five AM dependency categories, namely very highly dependent, highly dependent, moderately dependent, marginally dependent and independent. Accordingly, litchi can be classified as marginally to moderately dependent species.

Photosynthetic response of litchi seedlings to AM fungi

The promoted plant growth of litchi seedlings by AM fungi is normally attributed to enhanced nutrient uptake (Janos *et al*., 2001; Yao *et al*., 2005), however, nutrient status was not monitored in our study. Instead, the response of photosynthetic characteristics to AM fungal inoculation was investigated. Data demonstrated that AM fungal inoculation increased A_{max}, A_{qe} and LSP, and decreased LCP, but did not affected P_n. A_{max}, A_{qe}, LSP and LCP are photosynthetic parameters derived from fitting curve equation (Aleric and Kirkman, 2005), while P_n is photosynthetic parameter actually measured. Consequently, in our study, the higher photosynthesis potential as indicated by higher A_{max}, higher LSP and lower LCP for the inoculated rootstock seedlings can largely contribute to the increase in plant growth.

Enhanced photosynthesis by AM fungal inoculation has been extensively reported in many studies regardless of plant species (non-woody or woody plants) and growth conditions (non-stressed or stressed conditions) (Birhane *et al*., 2012; Zhu *et al*., 2012; Chen *et al*., 2014; Xiao *et al*., 2014). According to the published studies, photosynthetic rate was significantly increased by 14% due to AM fungal colonization on average (Kaschuk *et al*., 2009). In these reports, the parameters, P_n and/or A_{max}, were shown to increase with AM fungal inoculation. In our study, photosynthesis was promoted by AM fungi in terms of A_{max}, LSP and LCP, but not P_n. Meanwhile, the relationship analysis indicates that A_{max}, LCP and LSP seem more reliable than P_n in our study. In fact, the AM fungal effect on P_n was dependent on environmental factors, such as temperature and P level (Shrestha *et al*., 1995; Fay *et al*., 1996). For example, Shrestha *et al*. (1995) found that AM fungi enhanced P_n only at high temperature in August but did not affect it at relatively low temperature in September. Fay *et al*. (1996) indicated that AM fungal effect was dependent on P level, increasing A_{max} at low P level but decreasing it at high P level. In contrast, few works about AM fungal effect on A_{max}, LCP or LSP was reported so far. Boldt *et al*. (2011) demonstrated that AM fungal inoculation significantly increased A_{max}, LSP and decreased LCP, which is consistent well with the result in our study. However, Fay *et al*. (1996) indicated that AM fungal effect was dependent on P level, increasing A_{max} at low P level but decreasing it at high P level.

Mechanisms underlying the increased photosynthesis by AM fungi include improved nutrient status (Fay *et al*., 1996), enhanced carbon sink (see review by Kaschuk *et al*., 2009 and literature therein), promoted phytohormone level (Drüge and Schonbeck, 1993). The promoted uptake of P, K, Ca, Mg, Mn, Cu, Zn in litchi seedlings by AM fungi has been demonstrated (Janos *et al*., 2001; Yao *et al*., 2005) although the nutrient status was not monitored in our study. Inoculation of litchi rootstock seedlings with *Glomus intraradices* (namely *R. irregularis* in our study) or *G. margarita* increased IAA by 2-7 times and iPAs by 1.7-2.5 times (Yao *et al*., 2005). The increased phyotophormone levels by AM fungi may be fungal origin or plant origin induced by colonization (Ludwig-Müller, 2010). It can be speculated that the changes in phytohormones induced by AM fungal inoculation are probably responsible for the increased photosynthesis of litchi rootstock seedlings in our study.

Difference in the responses of two cultivars to AM fungi

It has been revealed that AM fungal inoculation effect depends on the rootstock genotypes of citrus (Graham *et al*., 1997). They found that the mycorrhizal dependencies of five genotypes were much different, with volkamer lemon (*Citrus volkameriana* Tan. and Pasq.) ranking the highest and trifoliate orange [*Poncirus trifoliate* (L.) Raf.] the lowest. In our study, only two litchi rootstock genotypes were estimated, however, they responded much differently to AM fungal inoculation. 'Heiye' showed positive response while 'Baila' negative. On one hand, this result indicates the necessity to screen appropriate rootstock (e.g. 'Heiye' in our study) in the grafting system of litchi if AM technology is considered. On the other hand, the difference in two fungal species should also be taken into consideration.

Our study was conducted in the nursery condition, and thus the obtained results can apply to the litchi propagation system. The positive response of 'Heiye' rootstock seedlings to AM fungi suggests the potential application in of AM fungi in litchi nursery. It is well recognized that AM fungal inoculation promoted the seedling growth in both grafting system (our study) and air-layer system (Janos *et al*., 2001; Sagar and Roy, 2013) due to improved photosynthesis (our study) or nutrient status (Janos *et al*., 2001). The successful application of AM fungi in propagation system of citrus has been well established for decades (Menge *et al*., 1982). For litchi, in the grafting propagation system using AM technology, 'Heiye' genotype should be the first choice as rootstock, however, evaluation with more genotypes is also recommended.

Conclusions

In conclusion, the inoculation of litchi rootstock seedlings with AM fungi affected the plant growth with differences between two genotypes in nursery condition. 'Heiye' responded positively while 'Baila' responded negatively to AM fungi. AM fungi increased the A_{max}, A_{qe} and LSP and decreased the LCP of 'Heiye', but did not affect those of 'Baila'. These differences could be responsible for the different growth responses of two genotypes to AM fungi. Due the prevalence of propagation with grafting in litchi nursery in China, our study highlights the potential application of AM technology in this system. However, precautions should be taken to select appropriate genotypes, and we recommend 'Heiye' according to the results in our study.

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