

Full Length Research Paper

# Impact of arbuscular mycorrhizal fungi on the growth and related physiological indexes of *Amorpha fruticosa*

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Through inoculating *Amorpha fruticosa* with arbuscular mycorrhizal (AM) fungi and without, the seedlings' growth indexes, dynamic characteristics of plant endogenous hormone levels, soluble sugar contents in roots and in leaves respectively, and physiological characteristics of seedlings utilizing nitrogen (N), phosphorous (P), were systematically studied. The results showed that both the two tested AM fungi strains, *Glomus mosseae* and *Glomus intraradices*, could colonize the *A. fruticosa* roots forming mycorrhizal complexes, and *G. mosseae* found to be the best AM fungus for *A. fruticosa* had a better colonization compatibility than *G. intraradices* on *A. fruticosa* roots with colonization percentage up to 88.5% that of the highest. On the one hand, inoculation with AM fungi could obviously elevate seedling heights, root collar diameter and dry biomass which were 1.94, 1.56 and 1.68 times that of control seedlings when *A. fruticosa* seedlings inoculated with *G. mosseae* at the last stage of seedling growth (110 days). On the other hand, the soluble sugar contents in roots and leaves of seedlings inoculated with AM fungi were significantly higher than control treatments, having a positive correlation with colonization percentage. In addition, AM fungi dramatically increased the contents of auxin (IAA), gibberellin (GA) and zeatin (ZR) in roots, but had little impact on abscisic acid (ABA). Furthermore, AM fungi inoculation not only improved per seedling total N, P contents, but also significantly increased the soluble N, P concentration of rhizosphere soil.

**Key words:** Arbuscular mycorrhizal fungi, *Amorpha fruticosa*, soluble sugar, endogenous hormone, N, P element.

## INTRODUCTION

*Amorpha fruticosa*, a perennial leguminous shrub plant, distributes mainly in vast majority regions of north of the Yangtze River and Huaihe River in China. It is an excellent soil and water conservation shrubbery, more importantly, its roots, stems and leaves contain Amorphenin, which is flavonoid glycoside with a variety of medicinal properties and hydrolysis of which can produce apigenin. Being excellent medicinal plants, the demands for *A. fruticosa* seedlings in recent years increase gradually and their cultivation research has become a hot.

Arbuscular mycorrhizal (AM) fungi are kinds of

endogenous mycorrhizal fungi that are widely distributed in nature. They can form symbiotic system with about 90% terrestrial plants (Graham, 2008). During the formation process of AM symbiosis, signal molecule exchanging between AM fungus and host plant occurs, which regulates various plant physiological indexes (Song and Song, 2011). The carbohydrates in the symbionts come mainly from plant photosynthetic products, especially glucoses that can be metabolized to release energy for maintaining all kinds of cellular activities and can also be used for synthesizing energetic materials, functional regulation substances and cellular structural components such as fats, proteins, celluloses and lignin, etc. Though with few sorts and extreme low levels, plant endogenous hormone involves nearly in all kinds of physiological and biochemical reactions throughout its biology processes from seed germination

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to plant aging. For example IAA plays a crucial role on plant growth, besides acting on cell elongation growth; it also plays a role on cellular division and cellular differentiation. The role GA play on plant growth resembles that of IAA, it not only promote cellular elongation, but also stimulate cellular division and cellular differentiation as well as plays a certain part on cambium differentiating to phloem and xylem. However, ZR as a kind of cytokinin obviously influences cellular division, mainly regulating cytokinesis, and absent of ZR can lead to cellular division deficiency and formation of multinucleated cells. While on the contrary, ABA can change the activity of some enzymes and has a inhibit effect on the whole plant growth. Studies have shown that AM fungi inoculation is strongly correlated with plant soluble sugar contents (Qi et al., 2000) and endogenous hormone levels (Jiao et al., 2006; Zhang et al., 2010). But little information is known about this correlation, and few papers have been published on the dynamic characteristics of soluble sugar contents and endogenous hormone levels of host plants and their relationships with colonization percentage when plants get association with AM fungi.

Pot-culture experiments were employed in this study to inoculate *A. fruticosa* with AM fungi, the growth response of *A. fruticosa* and their physiological indexes, the dynamic change characteristics of soluble sugar contents and, endogenous hormone levels with time, were investigated, which uncovered the promoting mechanism AM fungi have on *A. fruticosa* growth. The aim of our investigations was to provide partly theoretical basis for improving *A. fruticosa* production and quality.

## MATERIALS AND METHODS

The tested AM fungal strains, *G. intraradices* (GI) and *G. mosseae* (GM), are preserved in the Ecology Laboratory of Heilongjiang University, the inoculums of which with spore contents up to 1630/20 ml. The tested plant species is *A. fruticosa* provided by Jilin Provincial Academy of Forestry Sciences.

### Experimental design

Pot experiments were carried out in the greenhouse. The sets of experimental plants were divided into three main groups: one group inoculated with autoclaved inoculums, the other two groups inoculated with GI and GM respectively. All treatments had fifteen replicates; the total pots were forty-five, arranged in a random form.

### Seedling culture

*A. fruticosa* seeds were scarified with 20 to 30°C warm water, surface-sterilized with 0.3% K<sub>2</sub>MnO<sub>4</sub> for 3 to 4 h, and then germination was conducted less than 20 to 30°C for 60 h in an incubator after soaking for 24 h, the water was changed every other day. Culture matrix being a mixture of peat soil, sand and vermiculite at 5:2:3 ratios, was wrapped in brown paper autoclaved at 121°C, 0.1 MPa for 1.5 h, and then air dry a week ahead of the germinated *A. fruticosa* seeds were divided into the following three

treatments: (1) single inoculation with GI, inoculated with 60 g GI inoculums; (2) single inoculation with GM, inoculated with 60 g GM inoculums; (3) non-mycorrhizal or control plants (CK): with 30 g autoclaved (121°C, 0.1 MPa for 30 min) GI and GM inoculums addition respectively. The inoculums consisted of thoroughly mixed rhizosphere samples containing spores, hyphae and mycorrhizal root fragments. Plants were grown in a greenhouse (16 h photoperiod, 25/18°C day/night temperature, and 60% relative humidity) and watered three times per week. Base on the seedling growing periods and mycorrhizal formation characteristics, the plants were harvested and determined on the following items when the seedlings grew 30, 50, 70, 90 and 110 days.

### Mycorrhizal colonization percentage

Roots were washed free of soil and two subsamples were weighed, one of which was used for determination of other indexes and the other was cleared in 10% KOH and stained in Fuchsin blue by a modification of the method of Phillips and Hayman (1970), omitting phenol from the reagents and hydrochloric acid from the rinse. Percentage of root system colonized was calculated by means of the line-intercept method (Newman, 1966).

### Plant growth status

With 30, 50, 70, 90 and 110 days growth, seedling height and root collar diameter of 5 seedlings of each treatment were measured. Then the samplings were dried at 80 for 12 h for oven-dry biomass determination.

### Determination of soluble sugar contents

Anthrone-sulfuric acid colorimetric method was (Li, 2000) adopted for the determination of soluble sugar contents.

### Endogenous hormones

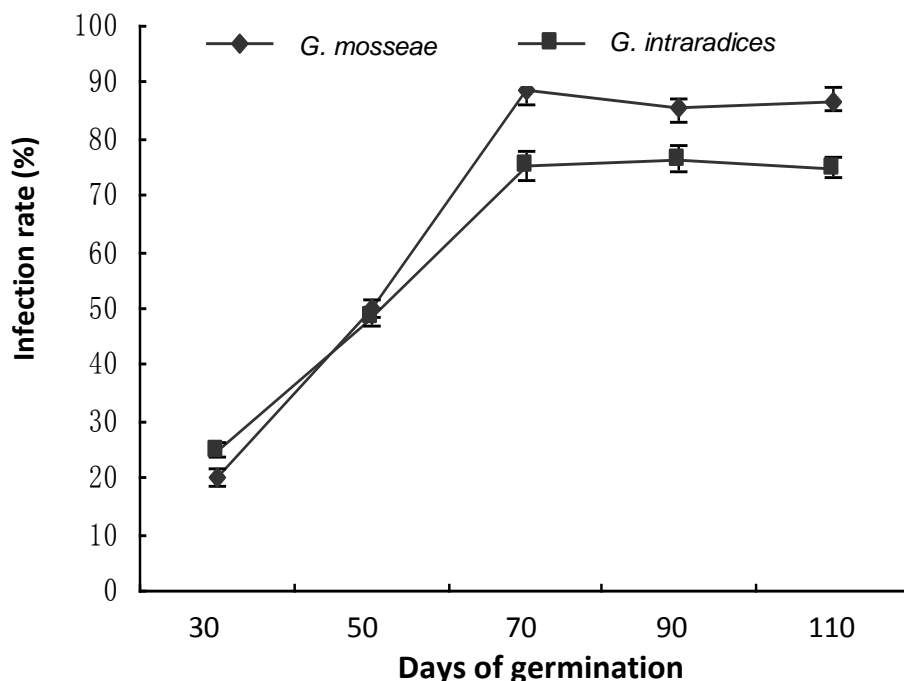
Plant endogenous hormone levels were determined by indirect Enzyme-LinkEd Immunosorbent Assay (ELISA), more information referred to Han et al. (2007).

### N, P levels in plants and in rhizosphere soil

When the seedlings grew into the last stage, that is, the 110<sup>th</sup> day, the seedlings were gently taken out together with roots. The soil attached to the surface of roots was determined to be rhizosphere soil. The total concentration of N in seedlings (1.5 g sample) and in the soil rhizosphere (4.0 g sample) was determined by the Kjeldahl method (Kjeldahl, 1883). Soluble nitrogen in the soil rhizosphere was examined by alkaline hydrolysis-diffusion method (Grasshoff, 1983). P was analyzed by a modified method according to Bowman (1989) after treatment with concentrated H<sub>2</sub>SO<sub>4</sub> and subsequent determination by spectrophotometry of phosphovanado-molybdate (yellow)-molybdenum blue, as described by Murphy and Riley (1962).

### Data processing

The data was subjected to analysis by Excel and SPSS13.0 statistical software. Analysis of variance (ANOVA) was carried out, followed by Duncan's Multiple Range Test ( $P < 0.05$ ) and correlation analysis to discriminate between the means.



**Figure 1.** The colonization percentage of *A. fruticosa* roots inoculated with *G. mosseae* and *G. intraradices*.

## RESULTS AND ANALYSIS

### Mycorrhizal colonization rates of seedlings

Both the two tested AM fungi, *G. mosseae* and *G. intraradices*, could form mycorrhiza with *A. fruticosa*. The infection law was alike as a whole, that is, the colonization percentage was low at the early stage and reached its peak when seedling growth at its vigorous (70 days), then with little changes afterward (Figure 1). However, significant differences between *G. mosseae* and *G. intraradices* on colonization percentage emerged at the vigorous phase and lasted to the end, suggesting that *G. mosseae* had a better compatibility with *A. fruticosa* compare with *G. intraradices*. No colonization was observed in the non-inoculated plant roots, implying the mixed soil before planting was autoclaved thoroughly and thereby enhancing the comparability of some physiological indicators between seedlings inoculated with AM fungi and CK.

### Influence of AM fungi inoculation on seedling growth

Among different treatments, each growth index showed the same change feature as the *A. fruticosa* seedling growing, that is, seedling height, root collar diameter and dry biomass increased gradually with time (Table 1). When the seedlings grew 30 days, significant differences ( $P < 0.05$ ) of seedling height and ground collar diameter between AM fungi inoculating treatments and that of

control had emerged, however, the differences of dry biomass did not show significant. With 70 days growth, seedling height between inoculated with *G. mosseae* and *G. intraradices* exhibited notable difference at  $P < 0.05$  level, but the dry biomass and root collar diameter between the two treatments did not show significant difference. After the seedlings grew 110 days, seedlings inoculated with *G. mosseae* displayed remarkable difference ( $P < 0.05$ ) with those inoculated with *G. intraradices* and those control seedlings in seedling height, root collar diameter and dry biomass. They exhibited superior growing trend, and their height, root collar diameter and dry biomass were 1.94, 1.56 and 1.68 times of control seedlings. Obviously, AM fungi have a promoting impact on *A. fruticosa* growing. Beside, seedlings had a great increase in height growth in the former 70 days, while during the 70 to 110 days, root collar diameter and biomass growth showed a big increase.

### Soluble sugar contents of host roots and leaves

When seedlings grew 30 days, there was no difference about apparent feature between mycorrhizal plants and control plants (Table 1). However, the soluble sugar levels of *A. fruticosa* in leaves were higher than in roots, which might due to the fact that leaves were the "workshop" of soluble sugar and could be called the "source" of soluble sugar, the carbohydrates in roots came mainly from the photosynthesis of leaves.

**Table 1.** Growth indexes of *A. fruticosa* seedling of different treatments on different stages.

Treatment		Time (days)				
		30	50	70	90	110
Height (cm)	GM	12.8±1.1 <sup>a</sup>	28.5±0.6 <sup>a</sup>	48.1±1.0 <sup>a</sup>	51.2±1.5 <sup>a</sup>	55.4±1.1 <sup>a</sup>
	GI	13.1±1.8 <sup>a</sup>	27.6±1.0 <sup>a</sup>	40.2±1.1 <sup>b</sup>	43.0±1.0 <sup>b</sup>	47.4±1.5 <sup>b</sup>
	CK	10.5±0.6 <sup>c</sup>	18.9±1.2 <sup>b</sup>	23.5±0.6 <sup>c</sup>	26.2±1.2 <sup>c</sup>	28.5±2.1 <sup>c</sup>
Root collar diameter (mm)	GM	0.9±0.1 <sup>a</sup>	1.5±0.2 <sup>a</sup>	2.7±0.3 <sup>a</sup>	4.8±0.5 <sup>a</sup>	6.4±0.6 <sup>a</sup>
	GI	0.9±0.2 <sup>a</sup>	1.4±0.2 <sup>b</sup>	2.6±0.6 <sup>a</sup>	4.7±0.3 <sup>a</sup>	5.2±0.8 <sup>b</sup>
	CK	0.8±0.1 <sup>c</sup>	1.3±0.0 <sup>c</sup>	1.8±0.5 <sup>b</sup>	3.0±0.8 <sup>b</sup>	4.1±0.7 <sup>c</sup>
Dry biomass (g)	GM	0.9±0.3 <sup>a</sup>	1.5±0.3 <sup>a</sup>	2.5±0.2 <sup>a</sup>	4.1±0.5 <sup>a</sup>	5.2±0.1 <sup>a</sup>
	GI	1.0±0.1 <sup>a</sup>	1.5±0.1 <sup>a</sup>	2.2±0.1 <sup>a</sup>	3.7±0.0 <sup>b</sup>	4.4±0.5 <sup>b</sup>
	CK	0.8±0.2 <sup>a</sup>	1.2±0.2 <sup>b</sup>	1.5±0.1 <sup>c</sup>	2.5±0.1 <sup>c</sup>	3.1±0.3 <sup>c</sup>

The values in the table are the mean of ten independent samples (values are mean ± SE, n=5). Different lowercase letters within the same column means significant difference ( $P<0.05$ ) (sample size = 5) on the same index among different treatments.

**Table 2.** Soluble sugar contents of sample inoculated with AM fungi or without (Units: mg/g).

Treatment		Time/day				
		30 days	50 days	70 days	90 days	110 days
GM	leaves	35.08±0.22 <sup>A</sup>	65.35±0.55 <sup>A</sup>	91.15±0.44 <sup>A</sup>	62.15±0.38 <sup>A</sup>	25.42±0.69 <sup>A</sup>
	roots	28.17±1.10 <sup>a</sup>	40.26±1.05 <sup>b</sup>	46.33±0.28 <sup>c</sup>	90.14±0.85 <sup>b</sup>	92.41±1.62 <sup>a</sup>
GI	leaves	36.11±0.98 <sup>A</sup>	60.96±0.36 <sup>B</sup>	86.57±0.82 <sup>B</sup>	64.12±0.67 <sup>A</sup>	24.13±0.56 <sup>A</sup>
	roots	27.28±0.45 <sup>a</sup>	38.97±0.68 <sup>b</sup>	50.26±0.71 <sup>b</sup>	95.47±1.04 <sup>a</sup>	94.16±0.41 <sup>a</sup>
CK	leaves	35.02±0.65 <sup>A</sup>	55.24±0.11 <sup>C</sup>	68.11±0.62 <sup>C</sup>	42.11±1.23 <sup>B</sup>	18.22±0.88 <sup>B</sup>
	roots	29.32±1.12 <sup>a</sup>	45.64±1.02 <sup>a</sup>	54.18±0.45 <sup>a</sup>	68.04±0.92 <sup>c</sup>	72.81±0.80 <sup>b</sup>

Standard Curve (CV):  $Y=0.0055X$ , herein Y is the reading value, X is sugar weight (mg),  $R^2=0.9994$ ; values in the table are mean ± SE (n=3); different lowercase letters within the same column mean significant differences exist in soluble sugar contents of plant roots at  $P<0.05$  level; different uppercase letters within the same column mean differences of soluble sugar contents of plant leaves are significant at  $P<0.05$  level.

low mycorrhizal colonization at this stage, in that mycorrhizal fungus had not become the competitor of primary roots of young plant for carbohydrates. When seedlings grew 50 and 70 days, the soluble sugar contents in mycorrhizal roots were strikingly lower than that of CK. Particularly at 70th day, soluble sugar levels in roots were much lower with GM treatment than with GI treatment. On the whole, soluble sugar contents in leaves inoculated with GM and GI were both dramatically higher than that of CK ( $p<0.05$ ). When the seedlings grew 90 days and 110 days, the two results in plants that were mycorrhizal were basically the same, and soluble sugar contents in both the roots and the leaves of the two mycorrhiza treating groups were obviously higher than control groups. Without doubt, the total amounts of soluble sugar in roots among all treatments increased with growth time extending.

### Endogenous hormone levels of host roots

Phytohormone balance is an important factor in regulating plant grow and development. In order to explored whether AM fungi involved in regulating endogenous hormone balance, we determined the contents in GI inoculating plants, GM inoculating plants and CK of four endogenous hormones, auxin (IAA), gibberellin (GA), zeatin (ZR) (a kind of cytokinin) and abscisic acid (ABA).

The IAA levels in different treated roots and at different growing stages are expected to be different with significance that can be obtained from Table 2, herein IAA levels arrived at the highest when the seedlings grow 70 days. IAA levels in plant roots inoculated with AM fungi were much higher than in CK roots all along the way, and in addition were much higher in GM treated roots

**Table 3.** Endogenous hormone levels of *A. fruticosa* roots inoculated with or without AM fungi (ng/g FW, herein FW is fresh weigh).

Treatment		Time (days)				
		30	50	70	90	110
Auxin (IAA)	GM	389.8±2.1 <sup>a</sup>	408.6±0.6 <sup>a</sup>	507.2±1.8 <sup>a</sup>	430.9±1.5 <sup>a</sup>	307.4±1.3 <sup>a</sup>
	GI	345.6±1.3 <sup>b</sup>	374.8±1.2 <sup>b</sup>	430.6±2.2 <sup>b</sup>	411.5±0.4 <sup>b</sup>	210.4±0.5 <sup>b</sup>
	CK	180.3±0.8 <sup>c</sup>	208.9±2.1 <sup>c</sup>	199.5±0.7 <sup>c</sup>	144.6±2.3 <sup>c</sup>	140.5±3.1 <sup>c</sup>
Gibberellins (GA)	GM	351.2±0.6 <sup>a</sup>	336.5±2.2 <sup>a</sup>	289.7±1.4 <sup>a</sup>	293.2±2.2 <sup>a</sup>	278.1±2.7 <sup>a</sup>
	GI	328.6±1.2 <sup>b</sup>	309.4±1.2 <sup>b</sup>	293.1±0.6 <sup>a</sup>	290.7±1.3 <sup>a</sup>	281.5±1.3 <sup>a</sup>
	CK	293.4±0.9 <sup>c</sup>	256.3±0.7 <sup>c</sup>	237.4±0.3 <sup>b</sup>	241.2±1.1 <sup>b</sup>	182.1±1.2 <sup>b</sup>
Zeatin (ZR)	GM	94.5±1.2 <sup>a</sup>	94.1±0.7 <sup>a</sup>	91.8±0.4 <sup>a</sup>	57.9±0.2 <sup>a</sup>	42.2±1.4 <sup>a</sup>
	GI	92.5±1.3 <sup>a</sup>	95.6±1.1 <sup>a</sup>	83.2±1.1 <sup>b</sup>	48.3±1.3 <sup>b</sup>	33.4±1.6 <sup>b</sup>
	CK	83.6±0.8 <sup>b</sup>	86.4±1.2 <sup>b</sup>	60.5±1.5 <sup>c</sup>	30.5±2.1 <sup>c</sup>	21.1±0.5 <sup>c</sup>
abscisic acid (ABA)	GM	114.5±1.8 <sup>b</sup>	186.7±1.9 <sup>a</sup>	147.9±2.1 <sup>a</sup>	269.5±1.1 <sup>a</sup>	298.8±2.7 <sup>a</sup>
	GI	112.6±2.4 <sup>b</sup>	191.2±3.1 <sup>a</sup>	151.1±1.5 <sup>a</sup>	271.1±0.6 <sup>a</sup>	301.5±1.6 <sup>a</sup>
	CK	129.7±0.8 <sup>a</sup>	185.5±2.3 <sup>a</sup>	149.4±0.8 <sup>a</sup>	268.3±2.5 <sup>a</sup>	303.8±1.9 <sup>a</sup>

The values in the table are mean ± variance (n=3); different lowercase letters on the same hormone in the table show significant difference ( $P<0.05$ ).

than in GI treated roots. These results showed that AM fungi could elevate the IAA levels of plant seedlings.

Via analyzing the GA and ZR levels of the seedlings on different stages, it was evident that the levels of GA and ZR were obviously low at the first 50 days of seedling growth and then decreased gradually afterward. With respect to GA, its levels in seedlings with GM treated were remarkably higher than that with GI treated ( $P<0.05$ ), but afterward of 70 days growth, this difference was removed from significance. As for ZR, the results were just the opposite, that is, the difference of ZR levels between GM seedlings and GI seedlings at the first 50 days was not so obvious, but at the beginning of 70<sup>th</sup> day, its levels in GM treated seedlings were clearly higher than in GI treated seedlings ( $P<0.05$ ). Throughout the whole growing period, the levels of GA and ZR in both AM fungi inoculated seedlings were higher than CK treatment ( $P<0.05$ ).

The data in Table 3 showed that ABA levels of roots in the three groups increased with time and reached its peak at the last stage of seedling growth, and only at the 30<sup>th</sup> day the values of ABA level in roots of GI and GM treated plants were remarkably higher than that of CK. While on other stages, ABA levels in the three treatments showed no significant difference. It is clear AM fungi inoculation has no impact on seedling root ABA levels.

#### Nitrogen, phosphorus levels in plants and rhizosphere soil

At the last phase of plant growth (110 days), Table 4 showed that N concentration in control group were higher

than that of AM fungi treated seedlings ( $P<0.05$ ), but as the dry biomass of AM fungi treated seedlings was markedly higher than that of control seedlings, so the total amounts of N in AM fungi treated plants were significantly higher than control plants ( $P<0.05$ ). Moreover, soluble N concentration in rhizosphere soil of mycorrhizal roots was dramatically higher than that of control ( $P<0.05$ ). On the other hand, although difference in plants among different treatments was not significant about P concentration, the amount of total P in AM fungi inoculated seedlings and soluble P levels in rhizosphere soil were both dramatically higher than control treatment at  $P<0.05$  level, and compared with *G. intraradices* inoculation, P level was higher when inoculated with *G. mosseae*. What these results indicated were that AM fungi could promote the uptake of N, P by host plants and at the same time elevated the available concentration of nutritional elements, suggesting AM fungi could mobilize the N, P that in immovable form into available form.

#### DISCUSSION

Both the two tested AM fungi strains *G. mosseae* and *G. intraradice* can form mycorrhizal complex with pot-cultured *A. fruticosa*, while *G. mosseae* has a better compatibility than *G. intraradices* with *A. fruticosa* roots. This consists with previous reports that the outcome (from positive to negative) for plant growth in different association varies (Smith et al., 2011; Smith and Smith, 2011). Colonization by different AM fungi does not result in the same growth responses in a single AM plant species

**Table 4.** N, P status in plants and rhizosphere soil with AM fungi inoculation or without.

Treatments	N concentration (%) of plants	Total N contents of plants (g/seedling)	Soluble N concentration of rhizosphere soil	P concentration (%) of plants	Total P contents of plants (g/seedling)	Soluble P concentration of rhizosphere soil
GM	1.7155 <sup>b</sup>	0.0892 <sup>a</sup>	118.325 <sup>a</sup>	0.2155 <sup>a</sup>	0.0112 <sup>a</sup>	152.361 <sup>a</sup>
GI	1.7004 <sup>b</sup>	0.0748 <sup>a</sup>	116.281 <sup>a</sup>	0.1984 <sup>a</sup>	0.0087 <sup>b</sup>	140.225 <sup>b</sup>
CK	1.8822 <sup>a</sup>	0.0583 <sup>b</sup>	90.624 <sup>b</sup>	0.2056 <sup>a</sup>	0.0064 <sup>c</sup>	119.362 <sup>c</sup>

Values in the table are mean±variance (n=3); different lowercase letters on the same physiological index show significant difference ( $P<0.05$ ).

(Klironomos, 2003; Munkvold et al., 2004; Smith et al., 2004). The outcomes of the symbioses are determined by interactions between plants and AM fungal genomes as well as environmental conditions, but the control mechanisms are still under the dark. Both the two AM fungi elevate the dry biomass of *A. fruticosa* seedlings (Table 1), and when seedlings grow 110 days, the correlation coefficient between dry biomass of seedling and colonization percentage is  $r = 0.96605$ , which implies dry biomass have positive relationship with colonization percentage and at the same time suggests *G. mosseae* can be made into microbial inoculums for large-scale production of *A. fruticosa* seedlings.

AM fungi colonization on *A. fruticosa* seedlings has a crucial impact on various physiological indexes of plants. The data in Table 2 can tell us that soluble sugar contents in leaves of seedlings inoculated with AM fungi are higher than in these of non-mycorrhizal seedlings, but reverse in roots. We speculate that the results are correlating to the special growth stage of seedlings. The first 70 days after sowing is the most productive time both for seedling growth and for AM fungi reproduction, at this time, AM fungi have the highest colonization rate. AM fungi are heterotrophic microorganisms, owning very little ability to live independently, and can only utilize simple carbohydrates provided by host plants for maintaining their life history. Even when AM fungi have formed symbionts with plants, they can not degrade host complicated carbohydrates (Kiers et al., 2011). Lauchli considered that mycorrhizal fungi consumed about 6 to 20% of the carbons fixed by photosynthesis (Olsson et al., 2010). Thus, primary roots of young seedlings compete for soluble sugars with infection hyphae of AM fungi, which lead to the lower soluble sugar levels in mycorrhizal roots than in CK. And these mycorrhizal complexes can be called the “gully” of carbohydrates. It is the formation of mycorrhizae that in turn affects other physiological indexes of seedlings, such as increases the leaf chlorophyll contents, enhancing their photosynthesis (Li et al., 2011), which are the bases of more carbohydrates been manufactured. What is more, the hosts increase soluble sugar production with plant growing, and their growth is furthermore promoted with improved mineral providing by symbiotic mycorrhizal fungi, and then host plants in turn promote the growth

and development of mycorrhizal fungi with improved carbohydrate supply. In the final phase of plant growth, soluble sugar contents in roots were much greater than in leaves and in mycorrhizal seedlings were higher than in CK. We believed that this was the real period that mycorrhizae increases soluble sugar contents in host plants, and mycorrhizal complex became the “pool” of soluble sugars. The reasons about the increase of soluble sugar contents in mycorrhizal roots in this study are not yet clear. Perhaps the aerial parts of mycorrhizal plants grow better than those of non-mycorrhizal plants, and then lots of carbohydrates manufactured via photosynthesis are translocated to roots, which may be a major cause of increase in soluble sugar levels.

Plant endogenous hormone plays a vital role on plant growth, development and production, and AM fungi in most case can improve their levels in mycorrhizal plants (Dugassa et al., 1996; Meixner et al., 2005). The latest studies also showed that AM fungi significantly increased the contents of IAA, GA and ZR in tomato roots (Yu et al., 2010), but not coincided with above data with respect to ABA. Therefore, different induced effects on ABA by AM fungi exist in different plants and the underlying inducing mechanisms necessity further investigation.

AM fungi cannot be pure cultured currently and thus whether they can synthesize phytohormone *in vitro* have not been confirmed (Bever et al., 2009; Parniske, 2008). Up to date, it is only confirmed that inoculated with AM fungi can increase the levels of some plant endogenous hormones in hosts. The elevated phytohormone synthesis may be contributed by improved carbohydrates providing adequate energy as well as well N, P supply. Whether these increased hormones are synthesized by AM fungus or by plant itself upon stimulated by AM fungus, or are contributed by both of the two, is subject to further investigation. In addition, who host plant or mycorrhizal fungus in hell is the determiner or the controller of mycorrhizal symbiosis formation, what role do different plant hormones play during mycorrhizal formation and what relationships between these hormones and symbiotic-related genes (Aroca et al., 2008) are all worth in deep exploring. In addition to these plant endogenous hormones, strigolactones, isolated as branching factors by Akiyama et al. (2005) in regulating AM symbiosis, is a novel class of plant hormone and their

synthesis is of IAA dependent (Brewer et al., 2009). Strigolactone and cytokinin (for example, ZR) acted antagonistically on bud outgrowth (Dun et al., 2012). The interaction of these plant endogenous hormones including these above mentioned and ethylene is very complex. To make matters worse, the list of hormones appears to be growing with new molecules such as bassinosteroids and jasmonates been added to the list. They synergistically or antagonistically work together and seem to be involved in modulating everything.

Nitrogen and phosphorous are the most important elements for plant growth, both are the component elements for nucleic acid and especially P is the core element for phospholipid which constructs the plasma membrane system. So life can not exist without N and P. Our study shows that AM fungi not only improve the N, P status of a single plant, but also can increase the rhizosphere soil soluble N, P concentration that is available for plants (Table 4). It may due to the fact that after AM fungus colonizing on a plant, its extracellular mycelium stretch a few centimeters from the root surface, which can sever as "elongation roots" to expend the absorption range of plant roots. In addition, extracellular mycelium may can secret some substances like organic acid for digesting the total N and P that can't be utilized by plants into soluble N and P that can be directly utilized by plants, thereby meeting the demands of plants for N, P. It is known that sufficient supply of nutrients is one of the most important factors for raising plant biomass.

In short, both *G. mosseae*, *G. intraradices* can dramatically promote *A. fruticosa* seedling growth, and during different growth and development stages of mycorrhizae, the corresponding change in plant physiological indexes including contents of soluble sugar which as one of photosynthetics, endogenous hormone levels and nutrient element contents occurs in hosts, which further reveals the promoting mechanism that AM fungi on plants and undoubtedly will provide a base for the application of AM fungi in the production of *A. fruticosa* seedlings.

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