

Arbuscular mycorrhizal fungi enhanced the growth, photosynthesis, and calorific value of black locust under salt stress

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Abstract

Saline soils spread wildly in the world, therefore it is important to develop salt-tolerant crops. We carried out a pot study in order to determine effects of arbuscular mycorrhizal fungi (AMF) (*Rhizophagus irregularis* and *Glomus versiforme*) in black locust seedlings under salt (NaCl) stress. The results showed that AMF enhanced in seedlings their growth, photosynthetic ability, carbon content, and calorific value. Under salt stress, the biomass of the seedlings with *R. irregularis* or *G. versiforme* were greater by 151 and 100%, respectively, while a leaf area increased by 197 and 151%, respectively. The seedlings colonized by *R. irregularis* exhibited a higher chlorophyll content, net photosynthetic rate, intercellular CO₂ concentration, stomatal conductance, and transpiration rate than that of the nonmycorrhizal seedlings or those colonized by *G. versiforme*. Both *R. irregularis* and *G. versiforme* significantly enhanced a carbon content, calorific value, carbon, and energy accumulations of black locust under conditions of 0 or 1.5 g(NaCl) kg⁻¹(growth substrate). Our results suggested that AMF alleviated salt stress and improved the growth of black locust.

Additional key words: arbuscular mycorrhizal colonization; biomass; carbon content; calorific value; saline conditions.

Introduction

Soil salinization spreads widely in the world; it is estimated that more than 6% of arable land suffers from salinity problem (Chaves *et al.* 2009). In China, there are about 10 million ha of salty soils (25% of agricultural land, Huang 2000). Due to imbalanced nutrient uptake, decreased antioxidant enzyme activities, and inhibited photosynthesis, a high salt content reduces crop productivity and quality (Yang *et al.* 2009, Sheng *et al.* 2011, Evelin and Kapoor 2014). As agricultural crops show low tolerance and poor growth under high salinity, salt-resistant trees were suggested to be planted on salty soils for their strong root systems and long life span (Wicke *et al.* 2011). With little demand of irrigation and fertilizer, which effectively reduces soils secondary salinization, salt-resistant trees plantation is beneficial for ecological restorations (Zhang *et al.* 2002a).

Black locust (*Robinia pseudoacacia* L.) is an important silvicultural tree species planted widely in temperate

terrestrial ecosystems around the world, and extensively in salty soils (Barrett *et al.* 1990, Zhang 2014). Black locust has attracted special attention in recent years as a potential species for bioenergy production because of its high biomass yield (Gasol *et al.* 2010) and calorific value (Carpenter and Eigel 1979). Zhang *et al.* (2010) found the biomass of black locust could reach 222.41 t per ha in salty soils of the Yellow River Delta in China. Studies conducted in a post-mining site reported that black locust produced 3–10 t per ha of dry biomass every year, which was much higher than that of poplar and willow at the same site (Bongarten *et al.* 1992, Grünewald *et al.* 2007, 2009). Black locust wood can be used as firewood or pyrolyzed into gas (Geyer and Walawender 1994), oil (Balat 2010) or ethanol (González-García *et al.* 2011, 2012). Calorific value is an important bioenergetic trait and a parameter for assessing energy plants (Kumar *et al.* 2011). Photosynthesis could influence the energy trait, which is measured

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Abbreviations: AMF – arbuscular mycorrhizal fungi; CE – carboxylation efficiency; Chl – chlorophyll; C_i – intercellular CO₂ concentration; E – transpiration rate; g_s – stomatal conductance; LA – leaf area; LDM – leaf dry mass; LSA – leaf special area; NM – nonmycorrhizal; NS – not salt-stressed; P_N – net photosynthetic rate; S – salt stress; WUE_i – intrinsic water-use efficiency.

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by the carbon content and calorific value (Kumar *et al.* 2011). However, there has been no report about the calorific value or carbon content in black locust under salt stress.

Arbuscular mycorrhizal fungi (AMF) are important microorganisms forming symbiosis with many plant species (Smith and Read 2008). AMF occur extensively in salty soils and are considered as suitable candidates for bioamelioration of saline soils (Evelin *et al.* 2009) and for improvement of plant tolerance to salinity (Porcel *et al.* 2012). AMF could decrease salt toxicity, enhance photosynthesis, and adjust ion osmotic potential of plants (Hajiboland *et al.* 2010, Selvakumar *et al.* 2014). Wu *et al.* (2010) reported that *Citrus tangerine* inoculated with

AMF showed a better ionic balance and higher photosynthetic rates under salt stress. Rewald *et al.* (2015) found that AMF could improve biomass accumulation of salt-stressed *Ulmus glabra* seedlings.

AMF could affect photosynthesis of trees in saline soils. However, there is little information about whether AMF could influence the calorific value and carbon content of black locust under salt stress. The main objectives of our study were (1) to reveal the effects of AMF on photosynthesis, calorific value, carbon content, energy and carbon accumulations in black locust seedlings under salt stress; (2) to compare the functions of *R. irregularis* and *G. versiforme* in improving the CO₂ fixation and bioenergy parameters of black locust under salt stress.

Materials and methods

Growth substrate and plant material: Soil [pH 7.5, soil/water ratio of 1:2.5 (m/v)] from the top soil layer (0–20 cm) was collected from campus of Northwest A&F University (Yangling, Shaanxi Province, China). Soil characteristics were as follow: 17.56 g(organic matter) kg⁻¹, 55 mg(available N) kg⁻¹, 19 mg(available P) kg⁻¹, and 232 mg(available K) kg⁻¹, which were measured according to the methods described by Bao (2000). Collected soil was air-dried and mixed with thoroughly washed river sand (sand:soil = 1:1, v/v). The mixture of soil and sand was autoclaved at 121°C for 2 h, and used as growth substrate.

Black locust seeds were collected in October 2011 from Northwest A&F University (Yangling, Shaanxi Province, China). Fully developed seeds were disinfected with 1% KMnO₄ for 10 min, and rinsed five times with sterile distilled water. Disinfected seeds were germinated on sterilized moist paper at 25°C in a plant growth chamber with 100 μmol(photon) m⁻² s⁻¹ light. Three germinated seeds were sown in each pot (diameter of 17 cm; depth of 16 cm) containing 1.5 kg of growth substrate, and thinned to one seedling per pot after 10 d.

AMF inoculation: *R. irregularis* (synonym of *G. intraradices* DAOM 197198) (Krüger *et al.* 2012) and *G. versiforme* (Karsten) Berch inocula used in this research were provided by the Bank of Glomales in China. The inocula comprised spores (about 50 spores g⁻¹), hyphae, infected root fragments, and sand. For mycorrhizal treatment, 20 g of the inoculum (either *R. irregularis* or *G. versiforme*) was applied next to the germinated seeds. For a nonmycorrhizal (NM) treatment, 20 g of the sterilized inoculum with 10 mL mycorrhizal fungal free filtrate meshing (through 1 μm nylon mesh) from AMF inocula was applied next to germinated seeds.

Experimental design: A preliminary experiment was conducted in order to select suitable salt concentrations for black locust seedlings. The seedlings were exposed to six salt concentrations, such as 0, 1.0, 1.5, 2.0, 2.5, 3.0

g(NaCl) kg⁻¹(growth substrate). The results (not shown) showed that 1.5 g(NaCl) kg⁻¹ caused obvious stress to NM black locust and greatly decreased its biomass. Mycorrhizal (AMF) seedlings showed no decrease in biomass, while other salt concentrations (2.0, 2.5, 3.0 g kg⁻¹) caused a damage to leaves or even seedling's death. This indicated that 1.5 g(NaCl) kg⁻¹ concentration caused obvious stress to NM seedlings, while it was suitable for the growth of AMF-treated black locust. The experiment consisted of a randomized block design with two factors: (1) AMF inoculation (the seedlings inoculated with *R. irregularis* or *G. versiforme*) and NM seedlings (control); (2) salt stress: 0 or 1.5 (S) g(NaCl) kg⁻¹(growth substrate). Each treatment had 5 replications. Salt stress was applied 80 d after sowing. In order to avoid osmotic shock, 20 mL of NaCl solution was successively added 5 times to each pot for every 2 d. The seedlings at 0 g(NaCl) kg⁻¹ (NS) were irrigated with distilled water. The pots were randomly arranged in a greenhouse with 12–14 h period of natural light, temperature of 12–35°C, and humidity of 40–85%. Each pot was weekly fertilized with 100 mL of half-strength Hoagland nutrient solution and watered with 200 mL of water.

Growth parameters: After 150 d of growth, stem heights and basal diameters (stem diameters at 1 cm above the soil surface) were measured, and seedlings were harvested for measurements of growth parameters.

Leaf area (LA) was determined using the disc method described by Gao (2006): 30 leaf discs were cut from the third, fourth, and fifth (from the top) fully expanded compound leaves and then dried to obtain the mass. The LA of the whole seedling was then calculated by determining the leaf dry mass (LDM) of the whole seedling and the leaf specific area (LSA cm² g⁻¹), which was LA = LDM × LSA.

Root, stem, and leaf dry mass were determined by drying the roots, stems, and leaves to constant mass in an oven at 80°C.

Mycorrhizal measurement: One hundred root fragments (about 1 cm) of each treatment were stained with trypan blue solution (Phillips and Hayman 1970). AMF colonization was measured by the method of Sun and Tang (2012).

Chlorophyll (Chl) content and gas-exchange: The Chl content was measured by using a *SPAD* Chl meter (*SPAD 502*, *Konica Minolta Sensing, Inc.*, Japan) according to the manufacturer's instruction. The terminal leaflet of 4th (from the top) fully expanded compound leaf of each black locust seedling was used to assay the Chl content and gas exchange. Gas-exchange parameters, including net photosynthetic rate (P_N), intercellular CO₂ concentration (C_i), stomatal conductance (g_s), and transpiration rate (E) were measured by using a portable open-flow gas exchange system *LI-6400* (*LI-COR*, Lincoln, NE, USA) from 08:30 to 11:30. The photosynthetically active irradiation was 1,000 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$, CO₂ concentration was 400 ppm, the temperature set in the chamber was 25°C, and the air flow rate was 0.5 $\text{dm}^3 \text{min}^{-1}$. Carboxylation efficiency (CE) and intrinsic water-use efficiency (WUE_i) were calculated as follows:

$$\text{CE} = P_N/C_i, \text{WUE}_i = P_N/g_s$$

Results

AMF colonization and plant growth: Colonization rates of *R. irregularis* were higher than that of *G. versiforme* at both NS and S [$1.5 \text{ g}(\text{NaCl}) \text{kg}^{-1}$] (Fig. 1). Application of S significantly reduced colonization of *R. irregularis* and *G. versiforme*. No AMF colonization was observed in the roots of NM black locust seedlings.

AMF increased the seedlings growth at both NS and S (Table 1). Under the NS condition, *R. irregularis* increased the root, stem, leaf, and whole plant biomasses of the seedlings compared with the NM seedlings by 72, 78, 99, and 80%, respectively. Under the S conditions, the increase was even higher compared with the NM seedling and equal to 130, 161, 184, and 151% in root, stem, leaf, and whole plant biomasses, respectively. *G. versiforme* increased the root, stem, leaf, and whole plant biomasses of black locust seedlings compared to NM seedlings by 50, 54, 66, and 55%, respectively. Under S stress, the increase of the root, stem, leaf, and whole plant biomasses were even higher compared with the NM seedlings by 83, 95, 146, and 100%, respectively. Both *R. irregularis* and *G. versiforme* significantly increased the seedling heights in NS soil, while under S conditions, only *R. irregularis* increased the heights and basal diameters of the seedlings compared to the NM seedlings. Without salt stress, only *G. versiforme* increased basal diameters of black locust seedlings compared to the NM seedlings. S stress

Carbon content and calorific value: The dry stems were ground to a fine powder that could pass through a 100-mesh or a 200-mesh sieve. The carbon content of dry stem (5 mg), which had been sieved through a 200 mesh, was determined using *Liqui TOCII* analyzer (*Elementar*, Germany) according to the manufacturer's instruction.

The gross calorific values of 1,000 mg of oven-dried powder (sieved through a 100-mesh sieve) of seedling stem was measured using an automatic isoperibol calorimeter (*OR2010*, China). Powder of 1,000 mg was inserted into a bomb with O₂ (2.0 MPa), and completely combusted in isoperibol calorimeter, where benzoic acid was used as a standard (calorific value = $26,470 \pm 20 \text{ J g}^{-1}$).

Contents of cell wall and lignin: Dry and ground (60 meshes) wood powder of 200 mg was used for cell wall and lignin content measurements using methods described by Luo and Polle (2009).

Statistical analysis: Data were subjected to analysis of variance (*ANOVA*) using *SPSS 16.0* (*SPSS*, Chicago, IL, USA), means by the *Duncan's* test and correlation by *Person's* two-tailed test were analyzed at the 5% level ($n = 5$). Figures were constructed with *Sigmaplot 12.0* (*Systat Software*, San Jose, CA, USA).

significantly decreased the growth of black locust seedlings, and reduced the root, stem, leaf, and whole plant biomasses of the NM seedlings and those colonized by *G. versiforme*.

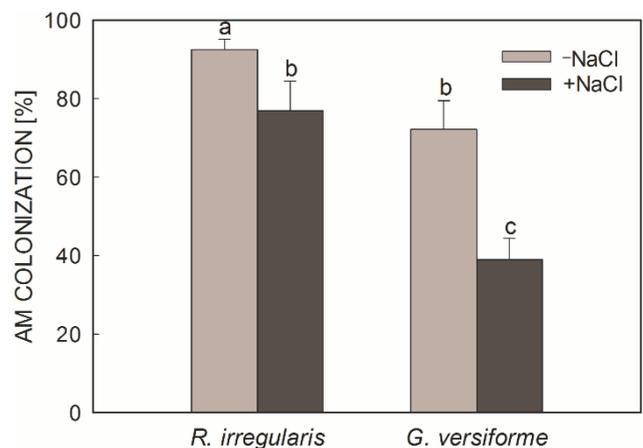


Fig. 1. The effects of NaCl and arbuscular mycorrhizal fungi (AMF) on root AMF colonization of black locust seedlings. NaCl salinity: -NaCl 0 g kg^{-1} and +NaCl 1.5 $\text{g}(\text{NaCl}) \text{kg}^{-1}$. AMF: *Rhizophagus irregularis* or *Glomus versiforme*. The data are mean \pm SD followed by different letters indicated significant difference at $p < 0.05$ by *Duncan's* test, $n = 5$.

Table 1. The effects of salinity and arbuscular mycorrhizal fungi (AMF) on dry mass, height, and basal diameter of black locust seedlings. Means (\pm SD) labeled with *different letters* within each column are significantly different ($p < 0.05$) by *Duncan's test*; $n = 5$. Levels of significance: * $p < 0.05$, ** $p < 0.01$.

Salinity [g kg ⁻¹]	AMF status	Dry mass [g plant ⁻¹]				Height [cm]	Basal diameter [mm]
		Root	Stem	Leaf	Whole plant		
0	Non-AMF	4.05 \pm 0.66 ^c	2.79 \pm 0.45 ^c	2.02 \pm 0.41 ^d	8.86 \pm 0.87 ^d	36.28 \pm 4.73 ^{cd}	5.40 \pm 0.26 ^b
	<i>R. irregularis</i>	6.98 \pm 0.68 ^a	4.97 \pm 0.12 ^a	4.03 \pm 0.38 ^a	15.98 \pm 0.79 ^a	49.74 \pm 6.61 ^a	5.82 \pm 0.42 ^{ab}
	<i>G. versiforme</i>	6.09 \pm 0.34 ^a	4.31 \pm 0.63 ^b	3.35 \pm 0.36 ^b	13.76 \pm 0.57 ^b	45.52 \pm 4.66 ^a	6.16 \pm 0.43 ^a
1.5	Non-AMF	2.76 \pm 0.30 ^d	1.73 \pm 0.23 ^d	1.11 \pm 0.27 ^e	5.59 \pm 0.62 ^e	30.50 \pm 5.83 ^d	5.41 \pm 0.85 ^b
	<i>R. irregularis</i>	6.36 \pm 0.62 ^a	4.51 \pm 0.12 ^{ab}	3.18 \pm 0.33 ^{bc}	14.05 \pm 0.28 ^b	43.22 \pm 2.93 ^{ab}	6.19 \pm 0.42 ^a
	<i>G. versiforme</i>	5.05 \pm 1.02 ^b	3.38 \pm 0.83 ^c	2.77 \pm 0.47 ^c	11.20 \pm 1.32 ^c	37.64 \pm 4.47 ^{bc}	6.04 \pm 0.42 ^{ab}
Significance							
Salinity		0.000**	0.000**	0.000**	0.000**	0.001**	0.637
AMF		0.000**	0.000**	0.000**	0.000**	0.000**	0.010*
Salinity \times AMF		0.508	0.352	0.588	0.200	0.893	0.543

Table 2. The effects of salinity and arbuscular mycorrhizal fungi (AMF) on chlorophyll (Chl) content, carboxylation efficiency (CE), intrinsic water-use efficiency (WUE_i), and leaf area of black locust seedlings. Means (\pm SD) labeled with *different letters* within each column are significantly different ($p < 0.05$) by *Duncan's test*; $n = 5$. Levels of significance: * $p < 0.05$, ** $p < 0.01$.

Salinity [g kg ⁻¹]	AMF status	Chl content	CE [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	WUE _i [$\mu\text{mol mol}^{-1}$]	Leaf area [cm ²]
0	Non-AMF	31.40 \pm 1.93 ^{cd}	0.031 \pm 0.005 ^{bc}	75.79 \pm 28.92 ^{ab}	478 \pm 97 ^d
	<i>R. irregularis</i>	39.46 \pm 2.76 ^a	0.043 \pm 0.004 ^a	69.32 \pm 11.63 ^{ab}	990 \pm 93 ^a
	<i>G. versiforme</i>	36.46 \pm 2.78 ^{ab}	0.042 \pm 0.003 ^a	67.82 \pm 13.50 ^b	777 \pm 83 ^b
1.5	Non-AMF	30.20 \pm 2.69 ^d	0.033 \pm 0.009 ^b	97.25 \pm 21.30 ^a	247 \pm 59 ^e
	<i>R. irregularis</i>	35.82 \pm 3.26 ^{ab}	0.037 \pm 0.005 ^{ab}	74.47 \pm 17.17 ^{ab}	734 \pm 77 ^b
	<i>G. versiforme</i>	34.50 \pm 3.58 ^{bc}	0.024 \pm 0.003 ^c	77.58 \pm 22.26 ^{ab}	620 \pm 105 ^c
Significance					
Salinity		0.041*	0.002**	0.110	0.000**
AMF		0.000**	0.009**	0.206	0.000**
Salinity \times AMF		0.631	0.003**	0.648	0.428

Chl content, CE, WUE_i, and LA: The AMF-treated seedlings had the higher Chl content, CE, and LA than that of the NM seedlings (Table 2). Without salt stress, LA of the seedlings colonized by *R. irregularis* and *G. versiforme* were 107 and 63% higher than that of the NM seedlings. Under salt stress, *R. irregularis* and *G. versiforme* increased LA of the seedlings by 197 and 151%, respectively. *R. irregularis* enhanced the Chl content, and *G. versiforme* increased CE. S and AMF had no significant influence on WUE_i of the black locust seedlings. S reduced the Chl content, CE, and LA of both the NM and AMF seedlings.

Gas exchange: The seedlings colonized by *R. irregularis* had higher P_N , g_s , C_i , and E than the NM seedlings under S, and only higher P_N under NS. The seedlings colonized by *G. versiforme* had higher P_N and E than that of the NM seedlings without stress. S did not affect P_N , g_s , C_i , and E of the NM seedlings (Table 3), while S decreased P_N of the seedlings colonized by *R. irregularis* or *G. versiforme*, and decreased g_s and E of the seedlings colonized by *G. versiforme*.

Calorific value, carbon content, cell wall, and lignin:

Both *R. irregularis* and *G. versiforme* enhanced the calorific value and carbon content regardless of salinity, and only increased their lignin content under NS conditions. *G. versiforme* increased the cell wall content in the seedlings (Table 4). S had negative effects on the calorific value, carbon content, cell wall, and lignin content, and significantly reduced the calorific value of the AMF and NM black locust stems.

Correlations between AMF colonization and calorific value and P_N :

There were positive correlations between the calorific value and the carbon content ($r = 0.602$, $p < 0.01$), or cell wall content ($r = 0.492$, $p < 0.01$), or the lignin ($r = 0.558$, $p < 0.01$) of black locust stem. The calorific value of black locust stem had significant correlations with P_N ($r = 0.662$, $p < 0.01$) or CE ($r = 0.537$, $p < 0.01$) of black locust seedlings (Table 5).

There were positive correlations between the calorific value and P_N with AMF colonization ($r = 0.659$, $p < 0.01$ or $r = 0.864$, $p < 0.01$, respectively).

Table 3. The effects of salinity and arbuscular mycorrhizal fungi (AMF) on net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), and transpiration (E) of black locust seedlings. Means (\pm SD) labeled with *different letters* within each column are significantly different ($p < 0.05$) by *Duncan's test*; $n = 5$. Levels of significance: * $p < 0.05$, ** $p < 0.01$.

Salinity [g kg ⁻¹]	AMF status	P_N [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	g_s [mol m ⁻² s ⁻¹]	C_i [$\mu\text{mol mol}^{-1}$]	E [mmol m ⁻² s ⁻¹]
0	Non-AMF	8.02 \pm 0.26 ^c	0.12 \pm 0.05 ^{ab}	263.43 \pm 45.47 ^{ab}	1.28 \pm 0.49 ^b
	<i>R. irregularis</i>	11.50 \pm 0.55 ^a	0.17 \pm 0.02 ^a	268.92 \pm 18.78 ^{ab}	1.76 \pm 0.22 ^{ab}
	<i>G. versiforme</i>	11.18 \pm 1.28 ^{ab}	0.17 \pm 0.06 ^a	267.00 \pm 18.73 ^{ab}	2.06 \pm 0.72 ^a
1.5	Non-AMF	7.35 \pm 1.11 ^{cd}	0.08 \pm 0.02 ^b	230.03 \pm 40.40 ^b	1.24 \pm 0.22 ^b
	<i>R. irregularis</i>	10.28 \pm 0.56 ^b	0.14 \pm 0.03 ^a	279.34 \pm 27.91 ^a	2.00 \pm 0.29 ^a
	<i>G. versiforme</i>	6.57 \pm 0.48 ^d	0.09 \pm 0.03 ^b	272.71 \pm 33.80 ^{ab}	1.29 \pm 0.29 ^b
Significance					
Salinity		0.000**	0.001**	0.632	0.226
AMF		0.000**	0.007**	0.149	0.009**
Salinity \times AMF		0.000**	0.209	0.273	0.030*

Table 4. The effects of salinity and arbuscular mycorrhizal fungi (AMF) on calorific value, carbon content, cell wall, and lignin of black locust stem. Means (\pm SD) labeled with *different letters* within each column are significantly different ($p < 0.05$) by *Duncan's test*; $n = 5$. Levels of significance: * $p < 0.05$, ** $p < 0.01$.

Salinity [g kg ⁻¹]	AMF status	Calorific value [kJ g ⁻¹]	Carbon content [%]	Cell wall [%]	Lignin [%]
0	Non-AMF	17.36 \pm 0.39 ^b	37.04 \pm 2.81 ^{cd}	83.14 \pm 1.68 ^{bc}	20.98 \pm 2.60 ^b
	<i>R. irregularis</i>	18.67 \pm 0.44 ^a	40.62 \pm 1.08 ^{ab}	85.79 \pm 2.04 ^{ab}	24.69 \pm 2.97 ^a
	<i>G. versiforme</i>	18.21 \pm 0.37 ^a	41.56 \pm 1.68 ^a	88.59 \pm 5.85 ^a	24.67 \pm 0.86 ^a
1.5	Non-AMF	16.61 \pm 0.27 ^c	34.62 \pm 2.34 ^d	79.78 \pm 2.22 ^c	19.79 \pm 0.99 ^b
	<i>R. irregularis</i>	17.49 \pm 0.34 ^b	38.05 \pm 3.56 ^{bc}	83.45 \pm 1.58 ^{bc}	22.22 \pm 0.73 ^b
	<i>G. versiforme</i>	17.28 \pm 0.48 ^b	39.96 \pm 1.07 ^{abc}	83.25 \pm 1.72 ^{bc}	21.92 \pm 0.93 ^b
Significance					
Salinity		0.000**	0.014*	0.002**	0.003**
AMF		0.000**	0.000**	0.007**	0.001**
Salinity \times AMF		0.464	0.878	0.516	0.584

Table 5. Correlations between calorific value and carbon content and cell wall content, lignin content, P_N , CE (*Pearson 2-tailed test* of significance). **. Correlation is significant at $p < 0.01$.

	Carbon content	Cell wall content	Lignin content	P_N	CE
Calorific value	0.602**	0.492**	0.558**	0.662**	0.537**

Carbon and energy accumulations in black locust stem:

Carbon and energy accumulations in stems of the NM seedlings were the lowest and suffered the greatest loss in contrast to the AMF seedlings. Both *R. irregularis* and *G. versiforme* greatly increased carbon accumulations in seedling stems at NS and S, with increment of 97 and 75%

at NS and 188 and 127% under S, respectively (Fig. 2). *R. irregularis* and *G. versiforme* increased energy accumulations by 92 and 63% under NS and by 175 and 105% under S (Fig. 3). Salinity significantly decreased carbon and energy accumulation in black locust stems.

Discussion

AMF could alleviate salinity stress and improve the growth and physiological status of plants (Porcel *et al.* 2012, Hameed *et al.* 2014). In our study, AMF significantly increased the biomass and P_N of black locust under salt stress. Importantly, we found that AMF enhanced the calorific value and carbon content of black locust in saline condition.

Colonization of AMF is a basis for improving growth

and physiologic status of plants under salt stress (Porcel *et al.* 2012). In this study, NaCl application significantly reduced colonization of *R. irregularis* and *G. versiforme*. Salt stress reduced AMF colonization, which was observed in many studies because high salinity suppressed AMF mycelium development (Sheng *et al.* 2008, Wu *et al.* 2010, Arafat and He 2011, Porcel *et al.* 2012). *R. irregularis* is a fast growing AMF and usually had higher

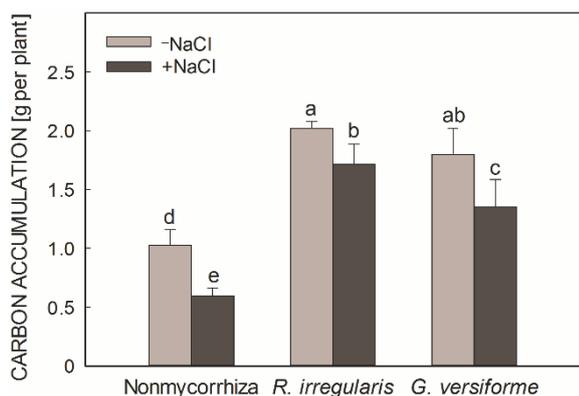


Fig. 2. The effects of NaCl and arbuscular mycorrhizal fungi (AMF) on carbon accumulations of black locust seedlings. NaCl salinity: $-NaCl$ 0 g kg^{-1} and $+NaCl$ $1.5 \text{ g(NaCl) kg}^{-1}$. AMF: nonmycorrhiza, *Rhizophagus irregularis* or *Glomus versiforme*. The data are mean \pm SD followed by different letters indicated significant difference at $p < 0.05$ by Duncan's test, $n = 5$.

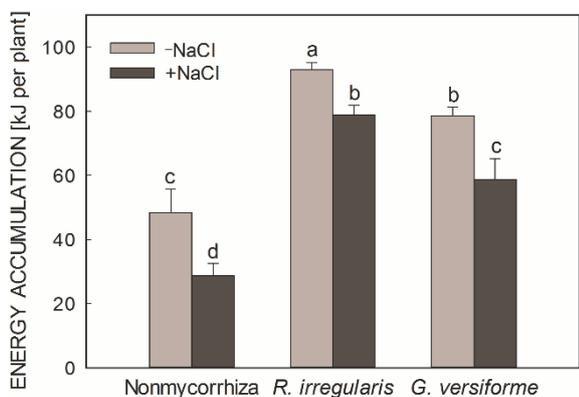


Fig. 3. The effects of NaCl and arbuscular mycorrhizal fungi (AMF) on energy accumulations of black locust seedlings. NaCl salinity: $-NaCl$ 0 g kg^{-1} and $+NaCl$ $1.5 \text{ g(NaCl) kg}^{-1}$. AMF: nonmycorrhiza, *Rhizophagus irregularis* or *Glomus versiforme*. The data are mean \pm SD followed by different letters indicated significant difference at $p < 0.05$ by Duncan's test, $n = 5$.

colonization rate than other AMF (Peng *et al.* 2011). Our results were consistent with previous studies, and *R. irregularis* showed the higher colonization rate than that of *G. versiforme* regardless of salt stress.

The biomass yield is an important parameter of salt resistance or tolerance of plants (Zhang *et al.* 2002b), as salty soils with a low water content could result in physiological drought and reduction of plant productivity (Evelin *et al.* 2009, Ruiz-Lozano *et al.* 2012, Hameed *et al.* 2014). In this study, both AMF significantly improved black locust growth under salt stress. This might attribute to AMF improving internal environment of plant cells through mineral nutrients uptake, osmotic regulation and plant water content increment (Sheng *et al.* 2008, 2011, Estrada *et al.* 2013, Evelin *et al.* 2013).

AMF-improved photosynthetic ability of plants also contributed to host growth. The Chl content is a key factor

reflecting the photosynthetic capacity (Takai *et al.* 2010, Zai *et al.* 2012). AMF increased the Chl content in plants due to higher phosphorus and magnesium content, and greater carbon sink strength in mycorrhizal seedlings (Kaschuk *et al.* 2009, Wu *et al.* 2010). In this study, both AMF increased the Chl content, P_N , and leaf area under salt stress, which was consistent with the study of Sheng *et al.* (2008), who found that AMF could enhance Chl contents and P_N of *Zea mays* under salt stress. The higher Chl content of mycorrhizal plants might lead to higher P_N and CE, which suggested that AMF plants had greater CO_2 assimilation rate with higher CE and greater leaf area. The presence of $1.5 \text{ g(NaCl) kg}^{-1}$ in soil did not influence the P_N , g_s , C_i , and E of the NM black locust seedlings in this study, since black locust was an important tree species for salty soils and possesses the tolerance to the high salinity (Zhang 2014). Generally, the carbon content (carbon/biomass, %) of a plant is constant, but it can be influenced by various factors, such as plant species, age, and ecological conditions. In this study, the increased carbon content of AMF-treated black locust seedlings might be due to the AMF-enhanced photosynthetic ability (Jongen *et al.* 1996). AMF enhancement of the carbon content would potentially have an impact on carbon sequestration. However, the carbon content (38–42%) of the mycorrhizal seedling stems in this study was lower than that in other hardwood species (46–50%) (Lamlom and Savidge 2003). Since mature trees have higher carbon content than younger trees (Prakash and Murray 1972, Kumar *et al.* 2011), the lower carbon content observed in this study might be due to the short period of the study (five months).

Carbon is a key nutrient element of organic matter. Therefore, higher carbon content in plants produced a higher calorific value (Zhu *et al.* 2014). In this study, both AMF species enhanced calorific value of black locust seedlings regardless of salinity. The significant increment of the calorific value in mycorrhizal seedlings might be due to the higher P_N and CE, which represent more solar energy absorption and more CO_2 assimilation, and would enhance carbon and lignin content of black locust seedlings (Demirbas 2005, Luo and Polle 2009, Fang *et al.* 2013).

Lignin is a main component of the plant cell wall, and a high lignin content of plants improves greatly their resistance to abiotic or biotic stresses (Bruce and West 1989). In this study, both AMF increased the cell wall and lignin content, and improved salt tolerance of black locust seedlings. The results showed that the lignin content was positively correlated with the calorific value ($r = 0.558$, $p < 0.01$), due to lignin having the high calorific value of 27 kJ g^{-1} (Luo and Polle 2009).

In summary, both *R. irregularis* and *G. versiforme* alleviated NaCl stress and increased biomass, photosynthesis, calorific value, carbon content, cell wall, and lignin content of black locust seedlings. Moreover, *R. irregularis* performed better than *G. versiforme* in

enhancing black locust growth, carbon, and energy accumulations. All these suggested that AM fungi,

especially *R. irregularis*, had great potential in enhancement of black locust seedlings biomass in saline soils.

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