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Research Article
Effects of Indigenous and Exotic *Rhizoglomus intraradices* Strains on Trifoliate Orange Seedlings

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Abstract
Background and Objective: Citrus plants are widely cultivated in tropical and subtropical countries and strongly depend on arbuscular mycorrhizal symbiosis. The present work was to evaluate the effect of indigenous and exotic *Rhizoglomus intraradices* strains on trifoliate orange for comparing the capacity of mycorrhizal fungi in citriculture. **Materials and Methods:** Two ecologic *R. intraradices* strains from China (indigenous) and Canada (exotic) were inoculated into potted trifoliate orange for 130 days. The root mycorrhizal colonization, root morphological traits, plant growth performance, soil glomalin concentrations, chlorophyll concentration and tissue nutrient levels were measured. **Results:** Mycorrhizal plants with indigenous *R. intraradices* strain had significantly higher root mycorrhizal colonization and entry points than those with exotic *R. intraradices* strain. Two *R. intraradices* strains collectively significantly increased plant growth performance, root morphology, chlorophyll concentrations and mineral nutrient levels compared with non-AMF treatments, whilst indigenous *R. intraradices* strain had superior effects than exotic *R. intraradices* strain. The AMF inoculation notably increased glomalin-related soil protein concentrations, whilst exotic *R. intraradices* strain had superior effects than indigenous *R. intraradices* strain. **Conclusion:** Indigenous *R. intraradices* strain conferred a superior role in trifoliate orange than exotic *R. intraradices* strain.

Key words: Arbuscular mycorrhiza, citrus, mineral nutrients, root architecture

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Citrus, a kind of worldwide cultivated fruit trees is a widely planted in the south region of China, which plays an important role in increasing farmers' income and stabilizing regional economic development. Citrus strongly depends on symbiotic mycorrhizas, namely, arbuscular mycorrhizas (AMs) to absorb nutrients and water from the soil[1]. Trifoliate orange (*Poncirus trifoliata* L. Raf.) is a citrus rootstock widely used in southeast Asia, which is greater dependence on AMs than other rootstocks[2].

The AMs are a symbiont between arbuscular mycorrhizal fungi (AMF) in the soil and roots of 80% terrestrial plants. The AMs are characterized by assisting the plant partner to absorb water and element nutrients[3]. It is well documented that AMF inoculation could stimulate nutrient absorption, enhance tree growth, improve root development and fruit quality, stabilize soil aggregates and increase the tolerance of abiotic and biotic stress in citrus plants[4]. In addition, AMs release a special glycoprotein, glomalin, into the soil, defined as glomalin-related soil protein (GRSP)[5]. In general, GRSP is insoluble in water and heat-stable under natural conditions and is considered as soil organic carbon source and the stabilizer in soil aggregates[6,7]. As a result, AMF would consider commercial use to rise crop yields and reduce fertilizer inputs. Commercial production of AMF inoculum is presently being attempted at USA, Canada, Australian and EU[8]. When these commercial AMF inoculums are sold in other countries, the exotic AMF species will face with the competition with the indigenous AMF species. However, the information regarding the comparative effects of exotic and indigenous AMF species on plants is considerably scarce.

The present study was to compare the effect of indigenous and exotic AMF species on plant growth, root development, mineral nutrients, GRSP production and chlorophyll levels of trifoliate orange seedlings.

MATERIALS AND METHODS

Plant set-up: The experiment was conducted between April 2, 2016 to August 19, 2016, in the glass greenhouse of the West Campus of Yangtze University, Jingzhou, China. The seeds of trifoliate orange were surface-sterilized with 70% of ethyl alcohol solutions for 10 min, rinsed two times with distilled water and germinated in autoclaved (0.11 MPa, 121°C, 2 h) sands at 26°C. After approx. one month, three-leaf-old trifoliate orange seedlings were transplanted into plastic pots (10 cm in depth, 15 cm in mouth diameter and 9 cm in inner diameter) containing 1.0 kg autoclaved (121°C, 0.11 MPa, 2 h) substrates of soils and sands (2:1, v/v) on April 2, 2016. After the time of transplanting, AMF inoculums including 1500 spores and infected root segments were mixed with the growth substrate. Non-AM fungal treatment was supplied with the autoclaved mycorrhizal inoculum as the control. All the seedlings were harvested after 130 days of AMF treatments.

Experimental design: The experiment consisted of three treatments in a completely randomized blocked arrangement: *Rhizoglomus intraradices* (N.C. Schenck and G.S. Sm.) Sieverd., G.A. Silva and Oehl from Canada (*R. intraradices* C AD), *R. intraradices* (N.C. Schenck and G.S. Sm.) Sieverd., G.A. Silva and Oehl from China (*R. intraradices* BJ) and non-AMF control. Each treatment had 6 replicates, leading to a total of 18 pots (three seedlings per pot).

Meanwhile, exotic *R. intraradices* was purchased from the Premier Tech. Ltd., (Myke FLR12), Avenue Premier, Riviere-du-Loup (Quebec), Canada. The Myke FLR 12 was used as the annual and perennial plant growth supplement. Indigenous *R. intraradices* (N.C. Schenck and G.S.Sm.) Schüßler and Walker (BGC JX04B) was isolated from Yujiang county, Jiangxi, China and provided by the Bank of Glomeromycota in China (BGC). The two *R. intraradices* strains were propagated by pot culture with white clover as the host plant for 3 months. The AMF inoculums contained spores, hyphae and infected root segments.

Determinations of variables: Plant growth-related parameters like plant height, stem diameter and leaf number per plant were determined before harvested. The seedlings were separated into the shoot and the root whose biomass was measured. After harvested, the roots of AMF and non-AMF-seedlings were carefully scanned by the Epson Perfection V700 Photo Dual Lens System (J221A, Indonesia) and the root figures were analyzed with a WinRHIZO professional software (Regent Instruments Inc., Quebec, Canada) in 2007 for root projection area, surface area, volume, total length and average diameter. Taproot length and the number of different order lateral roots were measured. Fresh 1 cm long root segments were cleaned by 10% (w/v) KOH solutions and stained with 0.05% (w/v) trypan blue by the protocol outlined by Phillips and Hayman[9].

The concentrations of soil GRSP fractions including easily extracted glomalin-related soil protein (EE-GRSP) and difficultly extracted glomalin-related soil protein (DE-GRSP) were assayed as per the method outlined by Wu *et al*.[10].

Leaf and root mineral nutrient (N, P, K, Ca, Mg, Fe, Mn, Cu and Zn) levels were determined by the Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-OES, American Thermoelectric Company, USA). Leaf chlorophyll levels were analyzed by the protocol described by Knudson *et al*.[11].

Statistical analysis: Data were analyzed by one-way variance (ANOVA) with SAS (SAS Institute Inc., Cary, NC, USA). The Duncan's multiple range (DMR) tests at $p<0.05$ was utilized to compare the significant differences between the treatments.

RESULTS

Root mycorrhizal status: Root mycorrhizal colonization and entry points in AMF-inoculated seedlings varied from16.4-46.1% and from 35-45, respectively (Table 1). Meanwhile, a considerably higher root mycorrhizal status was found under inoculation with indigenous *R. intraradices* than exotic *R. intraradices*.

Root morphology: In this study, AMF-inoculation was observed to alter different root traits of trifoliate orange seedlings. Indigenous and exotic *R. intraradices* markedly increased root total length, projected area, surface area and volume as compared with non-AMF treatment. However, there was no significant influence about taproot length and average diameter under *R. intraradices* versus non-AMF control (Table 2). As a whole, AMF-inoculated seedlings recorded a potential superior root figuration than non-AMF-inoculated seedlings. Indigenous and exotic *R. intraradices* treatments significantly increased the number of lateral roots, compared with non-AMF inoculation (Fig. 1). Meanwhile, indigenous *R. intraradices* strain exhibited a superior response on second and third-order lateral root number than exotic *R. intraradices* strain.

Plant growth performance: Compared with the non-AMF treatment, indigenous and exotic *R. intraradices* treatments significantly increased plant height, leaf number and leaf, stem and root biomass, with the exception of stem diameter (Table 1). Hereinto, leaf number, plant height and leaf biomass were notably higher under inoculation with indigenous *R. intraradices* strain than under inoculation with exotic *R. intraradices* strain.

Soil GRSPs changes: The present study showed significant differences in GRSP contents amongst the three treatments. AMF seedlings possessed significantly higher soil EE-GRSP and DE-GRSP concentration than non-AMF ones, regardless of indigenous and exotic AMF strains (Fig. 2). Exotic *R. intraradices* strain gave a 64.15 and 26.24% higher soil EE-GRSP and DE-GRSP concentration than indigenous *R. intraradices* strain.

Chlorophyll concentrations in leaves: Treatment with indigenous and exotic *R. intraradices* significantly increased leaf chlorophyll a, chlorophyll b and total chlorophyll concentrations in trifoliate orange seedlings, compared with non-AMF control (Fig. 3). Hereinto, indigenous and exotic *R. intraradices* significantly increased chlorophyll a levels by 62.31 and 7.25%, chlorophyll b levels by 62.32 and 11.69% and total chlorophyll levels by 62.32 and 9.5%, respectively. Based on the increased proportion, indigenous *R. intraradices* strain showed the superior effect on leaf chlorophyll levels than exotic *R. intraradices* strain.

Mineral nutrient levels in leaves and roots: Indigenous *R. intraradices*-inoculated trifoliate orange seedlings exhibited higher concentrations of N, P, K and Zn in leaves by 6.33, 100, 2.12 and 44.87% and higher concentrations of N, P, K, Ca, Mg, Fe, Mn, Cu and Zn in roots by 52.37, 126.15, 20.60, 47.39, 54.40, 43.16, 25.61, 205.91 and 74.25%, respectively, compared with non-AMF seedlings (Table 3). However,

Table 1: Effects of indigenous and exotic *Rhizoglomus intraradices* strains on root mycorrhizal development and plant growth of trifoliate orange seedlings

Treatments	Root colonization (%)	Entry point (No./cm)	Stem diameter (cm)	Plant height (cm)	Leaf number per plant	Biomass (g FW/plant) Leaf	Stem	Root
Non-AMF	0.0±0.0^{c}	0.0±0.0^{c}	0.235±0.019^{a}	11.72±0.72^{c}	16.5±0.6^{c}	0.188±0.010^{c}	0.376±0.024^{c}	0.491±0.020^{b}
Indigenous *R. intraradices*	46.1±2.4^{a}	45.0±2.0^{a}	0.245±0.020^{a}	17.98±1.42^{a}	22.3±1.3^{a}	0.404±0.034^{a}	0.644±0.041^{a}	0.859±0.045^{a}
Exotic *R. intraradices*	16.4±1.3^{b}	35.0±2.0^{b}	0.250±0.021^{a}	14.42±1.08^{b}	19.3±0.2^{b}	0.330±0.029^{b}	0.45±0.016a^{b}	0.834±0.046^{a}

Data (Means±SD, n = 6) followed by different letters indicated significant differences ($p<0.05$) between the treatments

Table 2: Effects of indigenous and exotic *Rhizoglomus intraradices* strains on root morphological traits of trifoliate orange seedlings

Treatments	Length (cm)	Project area (cm^{2})	Surface (cm^{2})	Diameter (cm)	Volume (cm^{3})	Taproot length (cm)
Non-AMF	164±6^{c}	7.9±2.0^{c}	24.7±1.5^{c}	0.59±0.02^{a}	0.29±0.03^{c}	20.5±0.3^{a}
Indigenous *R. intraradices*	301±28^{a}	15.2±0.8^{a}	48.9±3.2^{a}	0.48±0.03^{b}	0.60±0.06^{a}	22.5±1.4^{a}
Exotic *R. intraradices*	245±13^{b}	11.6±1.0^{b}	36.0±2.7^{b}	0.48±0.03^{b}	0.46±0.03^{b}	21.2±1.6^{a}

Data (means ± SD, n = 6) followed by different letters among treatments indicate significant differences (DMR, $p<0.05$) between the treatments

Table 3: Effects of indigenous and exotic *Rhizoglomus intraradices* strains on leaf and root mineral nutrient concentrations of trifoliate orange seedlings

Tissues	Treatments	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
		(mg g^{-1} DW)					(mg kg^{-1} DW)			
Leaf	Non-AMF	21.49 ± 0.67^{a}	0.86 ± 0.01^{b}	11.34 ± 0.19^{a}	16.21 ± 0.13^{a}	2.13 ± 0.02^{a}	373.51 ± 19.67^{a}	82.32 ± 0.3^{a}	1.62 ± 0.14^{b}	14.11 ± 1.32^{b}
	Indigenous *R. intraradices*	22.85 ± 1.47^{a}	1.72 ± 0.12^{a}	11.58 ± 0.21^{a}	16.21 ± 0.24^{a}	1.83 ± 0.03^{b}	294.25 ± 19.38^{b}	45.8 ± 1.54^{c}	1.7 ± 0.14^{b}	20.44 ± 3.02^{a}
	Exotic *R. intraradices*	21.49 ± 1.08^{a}	0.88 ± 0^{b}	11.69 ± 0.14^{a}	14.01 ± 0.03^{b}	1.85 ± 0^{b}	292.2 ± 15.95^{b}	53.59 ± 0.83^{b}	2.16 ± 0.18^{a}	15.72 ± 1.93^{b}
Root	Non-AMF	10.33 ± 0.51^{b}	0.65 ± 0.04^{b}	14.91 ± 0.32^{c}	5.17 ± 0.46^{c}	1.82 ± 0.10^{b}	3237 ± 347^{b}	231.43 ± 22.62^{b}	3.72 ± 0.12^{c}	22.21 ± 1.19^{c}
	Indigenous *R. intraradices*	15.74 ± 0.87^{a}	1.47 ± 0.08^{a}	17.98 ± 0.39^{a}	7.62 ± 0.45^{a}	2.81 ± 0.22^{a}	4635 ± 456^{a}	290.69 ± 24.53^{a}	11.38 ± 0.98^{a}	38.70 ± 1.51^{a}
	Exotic *R. intraradices*	11.59 ± 0.61^{b}	0.75 ± 0.04^{b}	16.84 ± 0.54^{b}	6.46 ± 0.39^{b}	2.07 ± 0.13^{b}	3599 ± 392^{b}	305.07 ± 21.81^{a}	6.19 ± 0.50^{b}	27.18 ± 2.40^{b}

Data (Means±SD, n = 6) followed by different letters among treatments indicate significant differences (DMR, $p<0.05$) between treatments

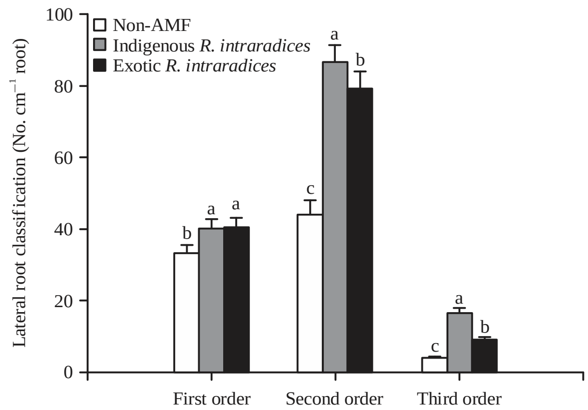

Fig. 1: Effects of indigenous and exotic *Rhizoglomus intraradices* strains on the number of different order lateral roots of trifoliate orange seedlings
Data (Means±SD, n = 6) followed by different letters among treatments indicate significant differences (DMR, $p<0.05$) between the treatments

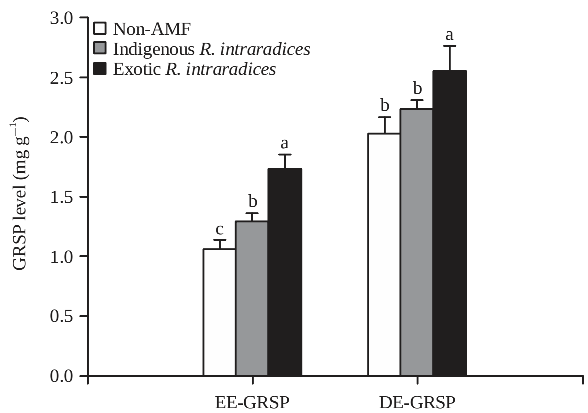

Fig. 2: Effect of indigenous and exotic *Rhizoglomus intraradices* strains on soil EE-GRSP and DE-GRSP concentration of trifoliate orange
Data (Means±SD, n = 6) followed by different letters among treatments indicate significant differences (DMR, $p<0.05$) between treatments

trifoliate orange seedlings inoculated with exotic *R. intraradices* only had 33.33% higher leaf Cu and 31.82% higher root Mn concentrations compared with those with non-AMF.

DISCUSSION

The present study indicated that the indigenous *R. intraradices* strain has relatively high efficient compatibility on root mycorrhizal development, plant growth performance and root morphology in trifoliate orange than the exotic *R. intraradices*. It seems that indigenous AMF strain showed strongly ecological adaption

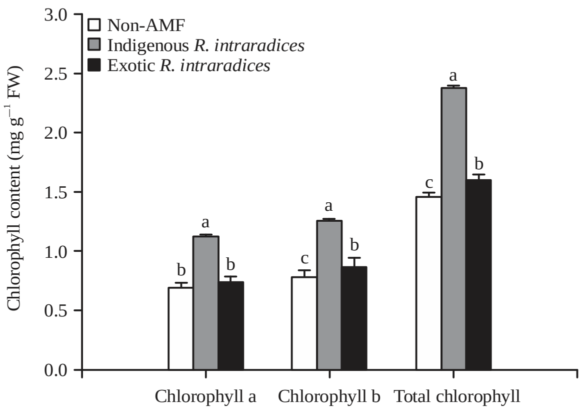

Fig. 3: Effects of indigenous and exotic *Rhizoglomus intraradices* strains on leaf chlorophyll a, chlorophyll b and total chlorophyll concentrations of trifoliate orange
Data (Means±SD, n = 6) followed by different letters among treatments indicate significant differences (DMR, p<0.05) between treatments

and roles in trifoliate orange than exotic AMF strain[12]. Such results can pointed out the importance of indigenous AMF in mycorrhizal application for sustainable agriculture and environment. The difference in functioning of AMF strains could probably be due to the differences in soil physiochemical properties and host identity.

In this study, AMF-inoculation in trifoliate orange represented better plant growth, root figuration and lateral root number than non-AMF-treatment. Meanwhile indigenous *R. intraradices* strain exhibited a superior response than exotic *R. intraradices* strain. The AMF-stimulated responses on roots would promote plant growth performance. Earlier studies also reported that inoculation with AMF could increase root total length, projected area, surface area and volume of alfalfa[13]. Further, mycorrhizal improvement of root systems was also observed under drought stress[14]. It suggested that mycorrhizal symbiosis can help the host plant to establish superior root system architecture in the soil interspaces for keeping a good contact with soils. Greater root systems under mycorrhization can explore more volume of soils for nutrient absorption. It implies that AMF-improved plant growth performance in host plants might be due to the capacity of AMs soil to help host plants absorbing water and nutrients from the soil through altering root system morphology (as seen in the study), gas exchange, leaf chlorophyll synthesis (as seen in the study) and developed soil hyphal network[15].

Studies had demonstrated the beneficial role of AMF in the formation and stabilization of aggregate stability via mycorrhizal hyphae and glomalin[16,17]. In this study, exotic *R. intraradices* strain conferred inferior root mycorrhizal colonization and root entry point number but superior EE-GRSP and DE-GRSP concentrations than indigenous *R. intraradices* strain. It is not consistent with Bedini *et al.*[18] in alfalfas inoculated with *Glomus mosseae* and *G. intraradices*. Possibly, in soils, GRSP extraction contains AMF and non-AMF proteins and other soil fungi and organic carbon are also extracted resulting in a mixture of GRSP in the extraction[10]. As a result, root mycorrhizal status is not the indicator of GRSP concentrations in soils. It indicated that mycorrhizal soils have the potential better soil fertility and soil aggregate stability than non-mycorrhizal soils.

In the present study, significantly higher leaf chlorophyll concentration was ranked as indigenous *R. intraradices*> exotic *R. intraradices*>non-AMF in the decreasing order. A similar result is also found by Wu *et al.*[19], who reported that AMF stimulated chlorophyll synthesis in host plants to maintain greater photosynthesis rate, which potentially provides more photosynthates for growth and development of both root systems and AMs.

Previous studies showed that mycorrhiza had a vital role in the absorption of mineral nutrients from the soil to the fungal partner[20]. The present study indicated that *R. intraradices* conferred a higher capacity in absorbing mineral nutrients of trifoliate orange than non-AMF treatment, whilst indigenous AMF exhibited better capacity in mineral absorption than exotic AMF. Such greater nutrient levels in AM plants would potentially provide the benefit in plant growth and gas exchange[21,22]. Greater nutrient levels in mycorrhizal plants may be due to the 2 explanations: (1) AM hyphae could directly absorb mineral nutrients[23] and (2) AM plants possess greater root architecture and root hair density, which potentially accelerate mineral nutrient absorption[24].

CONCLUSION

Mycorrhizal inoculation with *R. intraradices* could heavily improve plant growth performance, stimulate soil GRSP production and leaf chlorophyll concentrations, optimize root morphology and increase part mineral nutrients of trifoliate orange. Meanwhile, indigenous *R. intraradices* strain had strongly positive effects than exotic *R. intraradices* strain except for soil GRSP levels. Future studies will pay more attention to indigenous AMF than exotic AMF in the field of AMF biological researches.

SIGNIFICANCE STATEMENT

Mycorrhizal studies mainly focused on physiology, ecology, diversity and molecule levels, whereas the comparative effect of indigenous and exotic AMF strains is scarce. In this study, trifoliate orange seedlings were inoculated with indigenous (from China) and exotic (from Canada) *Rhizoglomus intraradices* strains in pots. It showed the positive effect of AMF on plant growth performance, root morphology, soil GRSP, leaf chlorophyll concentrations and mineral nutrients, whilst indigenous AMF strain exhibited superior capacity than exotic AMF strain. Such results can provide a clear path regarding indigenous AMF as a biofertilizer using in sustainable agriculture and environment.

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