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Research Article
Mycorrhizas Promote Plant Growth, Root Morphology and Chlorophyll Production in White Clover

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Abstract

Background and Objective: White clover generally possesses shallow root systems and is strongly dependent on mycorrhizas. This study was aimed to evaluate mycorrhizal roles in plant growth, roots and chlorophyll levels in white clover (*Trifolium repens*). **Methodology:** Three arbuscular mycorrhizal fungi (AMF) species, namely, *Rhizoglomus intraradices*, *Diversispora versiformis* and *Paraglomus occultum* were inoculated into potted white clover. **Results:** After 85 days of mycorrhizal inoculation, root mycorrhizal colonization of white clover ranged from 84.6-90.5%. Inoculation with AMF significantly increased leaf numbers, leaf area, petiole length and shoot and root biomass than non-AMF inoculation. Inoculated plants with *D. versiformis* and *P. occultum* recorded significantly higher *chlorophyll* a, b and a+b concentrations than non-inoculated controls. Mycorrhizal white clover plants had significantly higher nodule numbers, root total length, projected area, volume and number of 1st, 2nd and 3rd order lateral roots, as compared with non-mycorrhizal plants, regardless of AMF species used. **Conclusion:** It can be concluded that AMF has a positive contribution to promoting plant growth, root development and chlorophyll production in white clover. *Dive versiformis* represented relatively better effect than the other AM fungi in white clover.

Key words: White clover, arbuscular mycorrhizal fungi, root system architecture, biomass production

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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

Arbuscular mycorrhizal fungi (AMF) are a kind of beneficial microorganisms in soils, which are in a position to establish the symbiotic relationship with roots of most plants[1]. The AM symbiosis shows many benefits including uptake of nutrient and water, promotion of plant growth and enhancement of stressed resistance in the host plant[2,3]. Although experiments in the past showed a positive association between the host plant and AM fungi, the evidence regarding AM effects on root development is scarce[4].

Root system morphology refers to the spatial shape and distribution of straight roots or adventitious roots in growth substances[5,6]. In general, root systems have plastic and susceptible from all kinds of factors[7,8], such as soil microorganisms. Studies in the past had shown the AM fungal inoculation strongly stimulates the modification of root morphology in various plants[9-11]. In trifoliate orange, inoculation with AMF stimulated the formation of more fine roots and less coarse roots[10,11]. Isobe *et al.*[9] reported that inoculation with AMF represented a significant effect on the length and the number of tap roots and first and second-order lateral roots in kidney bean. Other studies also revealed the increase in the number of second-order roots and third-order lateral roots after inoculated with AMF[12,13]. These results suggested that AMF has a positive effect on root morphology.

White clover (*Trifolium repens* L.) can be characterized by higher productivity, faster regeneration and sprawl growth, popularly known to apply to plant greening[14,15]. However, white clover generally possesses shallow root systems in soils and often suffers from adverse conditions[16]. There are many beneficial microorganisms associated with white clover, such as AMF, etc[17]. The aim of the present study is to analyze the effects of three AMF species on plant biomass, chlorophyll content and root morphology of white clover plants.

MATERIALS AND METHODS

Plant culture: The seeds of white clover (*Trifolium repens* L.) were surface-sterilized with 70% for 10 min, rinsed three times with distilled water and sown into plastic pots (10 cm in depth, 15 cm in mouth diameter and 9 cm in inner diameter) containing 1.0 kg autoclaved (121 , 0.11 Mpa, 2 h) substance of soils and sands (2:1, v/v) on May 24, 2015. Each pot had 35 seeds of white clover, which was then thinned in 30 seedlings per pot after 5 days. After the time of sowing, 3000 spores of *Rhizoglomus intraradices*, *Diversispora versiformis* and *Poroglomus occultum*, provided by Bank of Glomeromycota in China (BGC) were mixed with the growth substance. Non-AM fungal treatment was supplied with the autoclaved mycorrhizal inoculum as the control.

The experiment consisted of four treatments in a randomized arrangement: *R. intraradices*, *D. versiformis*, *P. occultum* and non-AMF. Each treatment had three replicates, leading to a total of 12 pots.

Determinations of variables: The seedlings were harvested after 85 days of AM fungal treatments. All the plants were divided into shoots and roots, whose dry weight was determined after over-drying for 48 at 75°C. Each root system from these plants was scanned with an EPSON Flat-Scanner and analyzed with the WINRHIZO 2007d. The lateral root number in different orders was counted.

Root AM colonization was assessed by the protocol of Phillips and Hayman[18] with trypan blue. Chlorophyll concentration was measured by Knudson *et al.*[19] with the extraction of 80% acetone.

Statistical analysis: The data (Means±SD, n = 3) were statistically analyzed by one-way ANOVA in SAS (v8.1) and the Duncan's multiple range tests were used to determine the significance between these treatments at the 0.05 level.

RESULTS AND DISCUSSION

Root AM colonization: White clover plants could be heavily colonized by the three AMF species, varied from 84.6-90.5% in AM fungal colonization and 4.2-9.7 cm^{-1} root in entry points (Fig. 1). Meanwhile, inoculation with *D. versiformis* showed a relatively greater AM development in roots than other AM fungal treatments.

Plant growth performance: The present study showed that the inoculated white clover plants with AMF represented better leaf number, leaf area, petiole length and shoot and root biomass than non-AM plants (Table 1). Hereinto, *P. occultum* had more definite influence on these traits than the other AMF species. The result is in agreement with the findings of Wu *et al.*[20], who reported the positive effect of AMF on plant growth of peach (*Prunus persica*) plants. The improvement of plant growth in AM plants than in non-AM plants may be due to the fact that AMF is capable to help host plants to absorb more water and nutrients from the soil though developed extraradical haypae[21,22].

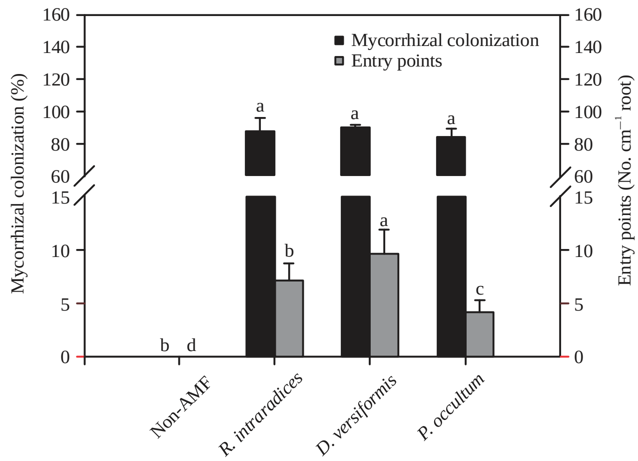

Fig. 1: Root colonization and entry point of white clover (*Trifolium repens* L.) seedlings inoculated with three AM fungi (*Rhizoglomus intraradices*, *Diversispora versiformis* and *Poroglomus occultum*)
Data (Mean±SD, n = 3) followed by different letters among treatment indicate significant differences at 5% level

Table 1: Effect of three AM fungi (*Rhizoglomus intraradices*, *Diversispora versiformis* and *Poroglomus occultum*) on growth performance and biomass production of white clover

Mycorrhizal treatments	No. of leaves per plant	Leaf area (cm^2)	Petiole length (cm)	Shoot weight (g DW plant^{-1})	Root weight (g DW plant^{-1})
Non-AMF	5.1±0.1^c	12.65±0.75^c	9.14±2.75^b	0.347±0.007^d	0.097±0.001^c
R. intraradices	6.8±0.2^b	13.61±0.46^c	10.14±2.41^b	0.533±0.024^c	0.201±0.017^b
D. versiformis	9.1±0.1^a	17.03±0.76^b	17.55±2.06^a	0.932±0.056^b	0.204±0.012^b
P. occultum	9.0±0.2^a	18.63±0.75^a	16.78±2.52^a	1.029±0.025^a	0.257±0.012^a

Data (Mean±SD, n = 3) followed by different letters among treatments indicate significant differences at 5% level

Table 2: Effects of three AM fungi (*Rhizoglomus intraradices*, *Diversispora versiformis* and *Poroglomus occultum*) on root morphological traits of white clover

Mycorrhizal treatments	Taproot length (cm)	No. of nodules per plant^{-1}	Total length (cm)	Projected area (cm^2)	Surface area (cm^2)	Average diameter (mm)	Volume (cm^3)
Non-AMF	12.7±0.3^b	21.5±1.0^d	91.7±6.7^c	7.11±0.60^b	12.89±0.15^b	2.74±0.08^b	0.061±0.000^d
R. intraradices	14.1±0.3^a	35.8±0.2^a	124.0±5.0^a	8.93±0.46^a	13.97±0.37^a	3.15±0.03^a	0.143±0.006^b
D. versiformis	13.2±0.3^b	28.7±0.9^c	118.4±0.3^{ab}	8.37±0.43^a	14.22±0.85^a	2.71±0.19^b	0.132±0.009^c
P. occultum	14.0±0.4^a	31.1±1.6^b	112.6±6.7^b	8.41±0.25^a	13.3±0.48^{ab}	3.03±0.08^a	0.156±0.002^a

Data (Mean±SD, n = 3) followed by different letters among treatments indicate significant differences at 5% level

Chlorophyll concentrations: The present results indicated that the treatment with *D. versiformis* and *P. occultum* but not *R. intraradices* significantly increased leaf chlorophyll a and chlorophyll a+b concentration in white clover seedlings, as compared with non-AMF inoculation (Fig. 2). In addition, all the AM treatments significantly increased chlorophyll b concentration, whereas did not significantly affect the level of leaf carotenoid, relative to non-AM treatment. The increase in leaf chlorophyll a, chlorophyll b and chlorophyll a+b under mycorrhization ranked as *D. versiformis*>*P. occultum*> *R. intraradices* in the decreasing order. This is consistent with the results in trifoliate orange by Wu *et al.*[23] and beach plum by Zai *et al.*[24]. Greater chlorophyll levels in AM plants suggested the more production of photosynthates in the host plant, which would provide more substrates for growth and development of root systems and AM symbiosis[25].

Root system morphology: In this study, inoculation with *R. intraradices*, *D. versiformis* and *P. occultum* dramatically increased root system morphological traits, including taproot length, total root length, surface area, volume and average diameter, relative to non-AMF control (Table 2, Fig. 3). In the three AMF species, *R. intraradices* and *P. occultum* had the superior effect on root morphology than *D. versiformis* (Fig. 3). It is in agreement with the results of Gutjahr *et al.*[26] in rice plants (*Oryza sativa*). Greater root system architecture in

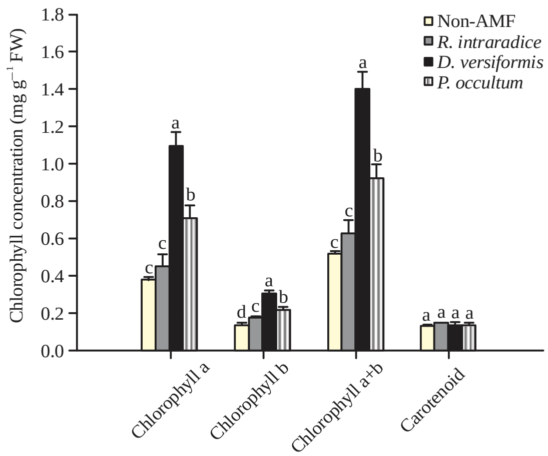

Fig. 2: Effects of inoculation with three AM fungi (*Rhizoglomus intraradices*, *Diversispora versiformis* and *Poroglomus occultum*) on chlorophyll concentrations of white clover (*Trifolium repens* L.) seedlings

Data (Mean±SD, n = 3) followed by different letters among treatment indicate significant differences at 5% level

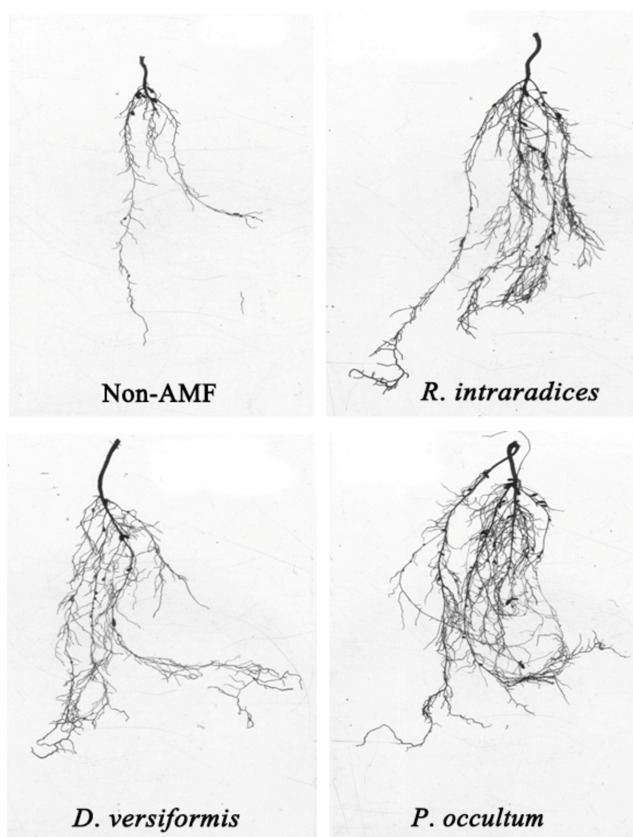

Fig. 3: Root morphology of white clover (*Trifolium repens* L.) seedlings inoculated with three AM fungi (*Rhizoglomus intraradices*, *Diversispora versiformis* and *Poroglomus occultum*)

AM plants will keep a good contact with soils, in favor of better uptake of water and nutrient[27,28].

Number of lateral roots: The present study showed that all the AM treatments significantly increased the number of first,

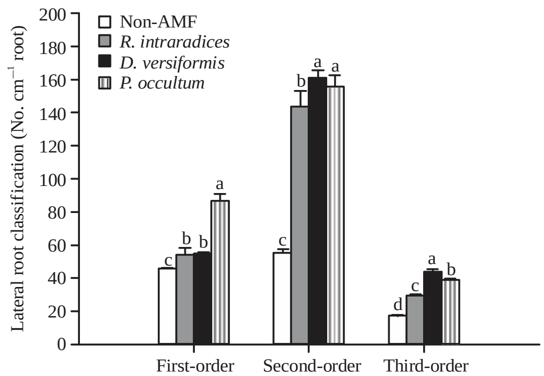

Fig. 4: Effects of inoculation with three AM fungi (*Rhizoglomus intraradices*, *Diversispora versiformis* and *Poroglomus occultum*) on the number of lateral roots in white clover (*Trifolium repens* L.) seedlings

Data (Mean±SD, n = 3) followed by different letters among treatment indicate significant differences at 5% level

second and third-order lateral roots, compared with non-AM treatment (Fig. 4). Number of first-order lateral roots in AM plants were 18.68, 21.10 and 91.43% significantly higher under *R. intraradices*, *D. versiformis* and *P. occultum* conditions than under non-AMF conditions. Mycorrhizal plants with *R. intraradices*, *D. versiformis* and *P. occultum* represented 160, 192 and 181% significantly higher number of second-order lateral root than non-mycorrhizal plants. The number of third-order lateral root was increased by 70, 155 and 127% respectively inoculating with *R. intraradices*, *D. versiformis* and *P. occultum* compared with non-AM fungal plants. This is in agreement with the findings of earlier studies in trifoliate orange and kidney bean[9,10,11]. Greater later root numbers in AM plants mean the plants contacting more soils, which is important for better plant growth.

CONCLUSION

As a consequence, the results showed that inoculation with all three AMF species significantly increased plant growth and biomass, promoted the production of chlorophyll a, b and a+b and stimulated better root morphological and lateral root formation and thus affect the growth and development of the white clover. Meanwhile, *D. versiformis* represented relatively better effect than the other AMF species in white clover.

SIGNIFICANCE STATEMENTS

- This study tried to evaluate the effects of three AMF species on white clover
- AMF inoculation significantly increased plant growth and shoot and root biomass
- AM white clover plants had significantly higher nodule numbers, root morphological traits and number of lateral roots as compared with non-mycorrhizal plants
- Inoculated plants with *D. versiformis* and *P. occultum* had higher *chlorophyll* a, b and a+b levels than non-inoculated controls

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