Effects of Arbuscular Mycorrhizal Fungi on Safflower Growth and Its Active Constituents: Postprint

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Abstract

Arbuscular mycorrhizal (AM) fungi are excellent microbial resources widely distributed in soil ecosystems that form symbioses with medicinal plants of important economic value. Under artificial cultivation conditions, this study conducted three AM fungal inoculation treatments on the traditional Chinese medicinal plant safflower (Carthamus tinctorius): Glomus mosseae, Glomus intraradices, and a mixed inoculum (G. mosseae, Glomus etunicatum, Glomus microagregatum, Glomus caledonium, Glomus cladoideum, and G. intraradices), with inoculation of an equal amount of inactivated inoculum serving as the control treatment. High-performance liquid chromatography was used to determine the changes in hydroxysafflor yellow A and kaempferol contents in safflower florets at different harvesting periods. The results showed that, compared with the control group, AM fungal inoculation treatments significantly promoted the accumulation of safflower biomass, and the mixed AM fungal inoculation effect was significantly higher than that of single AM fungal inoculation; in terms of secondary metabolite synthesis, there were no significant differences in hydroxysafflor yellow A and kaempferol contents in safflower between AM fungal inoculation treatments and different harvesting periods; indicating that although AM fungi can promote safflower growth, they do not affect the synthesis of hydroxysafflor yellow A and kaempferol.

Full Text

Effects of Arbuscular Mycorrhizal Fungi on Growth and Active Constituents of *Carthamus tinctorius*

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Abstract

Arbuscular mycorrhizal (AM) fungi represent excellent microbial resources widely distributed in soil ecosystems and are frequently utilized in medicinal plants with significant economic value. In this study, *Carthamus tinctorius* was inoculated with different AM fungal species: *Glomus mosseae*, *Glomus intraradices*, and a mixed AM inoculum (*G. mosseae*, *G. etunicatum*, *G. microaggregatum*, *G. caledonium*, *G. claroideum*, and *G. intraradices*), with an equivalent amount of inactivated bacteria serving as the control group. The inoculation effects of AM fungi were evaluated by measuring infection rates and soil spore density, while the contents of hydroxysafflor yellow A (HSYA) and kaempferol in *C. tinctorius* florets were determined via high-performance liquid chromatography at different harvesting stages. The objectives were to investigate the effects of AM fungi on the growth and active constituents of *C. tinctorius* and to analyze how different flowering stages influence HSYA and kaempferol accumulation, thereby providing an important theoretical basis for promoting agricultural production of *C. tinctorius* in Xinjiang. The results demonstrated that AM fungi significantly promoted the accumulation of aboveground biomass, root biomass, and floral biomass in *C. tinctorius*. However, regarding secondary metabolite synthesis, no significant differences were observed in HSYA or kaempferol contents across different AM fungal treatments or growth stages.

Keywords: arbuscular mycorrhizal fungi; mycorrhizal infection rate; soil spore density; secondary metabolites

1. Materials and Methods

1.1 Study Site and Experimental Design The experimental site was located at Shihezi University (85°59 44 E, 44°19 34 N, altitude 404 m). The region features an arid climate with annual precipitation of 125–208 mm, mean annual temperature of 6.9°C, and a frost-free period of $’ (cid:230)fn (cid:211)(cid:212)。_’ U89op: pH7.9、(cid:146)q(cid:181) 9.15%、rs 0.55g·kg−1、rt 0.26g·kg−1、ru 31.29g·kg−1、v (cid:236) s 33.83mg·kg−1、J (cid:127) t 24.29mg·kg−1、w(cid:127)u 526.06mg·kg−1、Jx(cid:215) 28.0g·kg−1。

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*!“#¨。Q_* AM *644o*: *fghij* (Glomus *t“(cid:135)(cid:136)o(* AM *64poyzKC&*,

+~(cid:127) mosseae) l5khij (Glomus intraradices) {|} (cid:128)(cid:129)(cid:130)(cid:131)(cid:132)L(cid:133)ab(cid:140)=(cid:141)Vab(cid:142)(cid:130)(cid:143) A l(cid:144) ~(cid:127)d_ ‘(cid:128) (cid:216) Q, m n 4 o (G. mosseae, Glomus EFGH: 2018−08−30; IJGH: 2018−11−14 KLMN: (cid:132)(cid:133)(cid:134)(cid:135)(% V (cid:136)(cid:137)(cid:218) (31560656, 31871568); , - . / %) X (cid:138) (cid:139) d (cid:137) (cid:218) (YJRC2017−3); ! ” # $ days. The experiment employed a completely randomized design with four treatments: control (CK, inactivated bacteria), *G. mosseae* (M1), *G. intraradices* (M2), and mixed AM fungi (M3). Each treatment comprised four replicates.

1.5 Inoculation and Cultivation Sterilized substrate (300 g soil and 250 g sand per pot, mixed 1:1:1) was autoclaved at 121°C for 2 h, with a second sterilization cycle applied after 24 h. The substrate moisture content was maintained at 80%, with growth chamber conditions set at 23°C and a 8h light/16h dark photoperiod. The cultivation period lasted 60-75 days at 25-33°C with 20-25% relative humidity. After 75 days, plant biomass and mycorrhizal colonization parameters were measured. All treatments received Hoagland nutrient solution weekly at a 1:100 dilution, with nutrient levels maintained at 65-75% of field capacity.

1.5.2 HPLC Determination of Active Compounds Hydroxysafflor yellow A analysis followed the method described in reference [16]. Dried floret samples (0.4 g) were extracted with 25 mL of 25% ethanol, ultrasonicated for 40 min, and filtered. HPLC conditions: Agilent C column (4.6 mm × 250 mm, 5 m); mobile phase of methanol:acetonitrile:0.7% phosphoric acid (26:2:72); detection wavelength 403 nm; flow rate 1.0 mL · min^{1}; column temperature 30°C; injection volume 20 L.

Kaempferol analysis followed reference [17]. Samples (0.5 g) were hydrolyzed with 25 mL of 25% hydrochloric acid, refluxed for 30 min, then extracted. HPLC conditions: Agilent C column (4.6 mm × 250 mm, 5 m); mobile phase of methanol:0.4% phosphoric acid (52:48); detection wavelength 360 nm; flow rate 1.0 mL · min^{1}; column temperature 35°C; injection volume 10 L.

2. Results

2.1 Mycorrhizal Infection Rate and Spore Density AM fungal inoculation significantly increased both infection rates and soil spore densities compared to the control ($P < 0.05$). Infection rates in M1, M2, and M3 treatments increased by 168%, 188%, and 239%, respectively. Spore density followed similar trends, with M3 showing the highest colonization intensity. No significant differences were observed between M1 and M2 treatments ($P > 0.05$), though both differed markedly from CK and M3 ($P < 0.05$) [Figure 2: see original paper].

2.2 Plant Biomass Accumulation AM fungi significantly enhanced both aboveground and root dry weights ($P < 0.05$) [Figure 3: see original paper]. The promotion effects ranked as M3 > M2 > M1 > CK. At 117 days post-inoculation, M1, M2, and M3 increased total biomass by 32%, 95%, and 194%, respectively, compared to CK. Strong positive correlations existed between mycorrhizal infection rates and plant biomass components ($r > 0.85$, $P < 0.01$) [Figure 4: see original paper].

2.3 Effects on Active Constituents Despite biomass improvements, AM fungal treatments did not significantly affect HSYA or kaempferol concentrations in florets ($P > 0.05$) . HSYA content remained stable across all treatments (0.086–0.090 mg · g ^{1}), while kaempferol showed minor variations without statistical significance. Different growth stages also failed to produce significant differences in active compound accumulation [Figure 5: see original paper].

TABLE:1 Effect of AM fungi on dry weight yield, hydroxysafflor yellow A, and kaempferol in *C. tinctorius*

Treatment	Dry Weight (g · plant ^{1})	HSYA Content (mg · g ^{1})	Kaempferol Content (mg · g ^{1})
CK	6.318 ± 0.14 d	0.0897 ± 0.010 a	0.2393 ± 0.011 a
M1	9.323 ± 0.19 c	0.0863 ± 0.010 a	0.2443 ± 0.018 a
M2	10.049 ± 0.22 b	0.0890 ± 0.009 a	0.2516 ± 0.019 a
M3	17.819 ± 0.45 a	0.0860 ± 0.011 a	0.2389 ± 0.016 a

Note: Different lowercase letters indicate significant differences at $P < 0.05$ *level.*

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3. Discussion

AM fungi establish mutualistic symbioses with most terrestrial plants, enhancing nutrient uptake and stress tolerance 〔7-9〕. Previous studies demonstrated that AM colonization significantly improves biomass accumulation in medicinal plants such as *Leymus chinensis* 〔10〕, *Lilium brownii* 〔11〕, and *Erodium oxyrhynchum* 〔12〕. Our results corroborate these findings, showing that mixed AM fungal inoculation (M3) produced the most pronounced growth-promoting effects, increasing *C. tinctorius* biomass by 182% compared to CK.

The absence of significant effects on HSYA and kaempferol contents aligns with previous reports on secondary metabolite regulation by AM fungi 〔18-20〕. While AM symbiosis can enhance the production of certain phytochemicals through improved nutrient acquisition 〔21-24〕, the biosynthesis of flavonoids and quinochalcones in *C. tinctorius* appears primarily governed by genetic factors and developmental stage rather than mycorrhizal status. Studies on *Glycyrrhiza uralen-*

sis [25] and *Hypericum perforatum* [29] reported similar genotype-dependent responses, where AM effects on active compounds varied among species and metabolite classes.

The growth-stage independence of metabolite accumulation observed here contrasts with some reports [30-32] but supports the hypothesis that *C. tinctorius* maintains relatively stable secondary metabolite profiles throughout flowering. This characteristic is advantageous for standardized medicinal production, ensuring consistent active compound content regardless of harvest timing within the flowering period.

4. Conclusion

AM fungal inoculation, particularly with mixed species, substantially enhances *C. tinctorius* biomass without compromising the quality of active constituents. While mycorrhizal symbiosis effectively promotes plant growth in Xinjiang's arid conditions, it does not significantly alter HSYA or kaempferol concentrations. These findings provide practical guidance for cultivating high-yield, quality-stable safflower in arid regions, suggesting that AM fungi can be integrated into agricultural systems to improve productivity while maintaining medicinal efficacy.

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Note: Figure translations are in progress. See original paper for figures.