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AM fungi effects on the growth and physiology of *Zea mays* seedlings under diesel stress

M. Tang [a,*], H. Chen [a], J.C. Huang [b], Z.Q. Tian [c]

[a] College of Forestry, Northwest A&F University, Taicheng Road 3, Yangling, Shaanxi 712100, China
[b] College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, China
[c] Foreign Language Department, Northwest A&F University, Yangling, Shaanxi 712100, China

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ABSTRACT

The effects of an arbuscular mycorrhizal fungus (AMF) (*Glomus constrictum* Trappe) on the growth and some physio-biochemical indexes of *Zea mays* L. seedlings under different levels of diesel stress were investigated in a pot study. Generally, the symbiotic relationship between corn and AMF can be well established under diesel stress. This was reflected by the better physio-biochemical index of the plants inoculated with *G. constrictum* whose colonization rates were between 47.30% and 91.50%. Compared with the non-inoculated ones, the heights and basal diameters of the inoculated seedlings increased by 0.08–47.20% and 6.74–35.71% respectively. The relative contents of chlorophyll and soluble proteins increased by 1.88–38.79% and 3.87–77.27% respectively, while the contents of malondialdehyde and free proline decreased by 2.74–52.74% and 24.69–32.86%. Three antioxidant enzymes reacted differently under the diesel stress. The activities of superoxide dismutase (SOD) and catalase (CAT) increased at low diesel concentration, but decreased at high concentration. In contrast, peroxidase (POD) had a decreased activity at low diesel concentration, but an increased activity at high concentration. On the whole, the activity of three antioxidant enzymes in the plants inoculated with AMF were higher than those without AMF inoculation. Our results support the view that antioxidant enzymes have great influence on the biomass of plants, and AMF can improve the capability of scavenging the reactive oxygen and alleviate *Z. mays* seedlings from diesel stress.

1. Introduction

Arbuscular mycorrhiza (AM), the symbiont of arbuscular mycorrhizal fungi (AMF) and host plant root, has proved to be able to improve soil structure and enhance plant resistance to environmental stress (Tang and Chen, 1999). Recently, the enhanced degradation of organic pollutant in soils in the presence of AM was observed, indicating that arbuscular mycorrhizal bioremediation is a promising and prospective technique for soils contaminated with organic pollutant (Li et al., 2006). Contrast to the traditional remediation methods for contaminated soils, bioremediation is an economically non-destructive approach (Huang et al., 2002). The mycorrhiza can improve plant growth by enhancing mineral nutrition absorption and resisting various stresses, and can degrade or transfer organic pollutant. Over the last few years, the use of AMF as a potential biological control agent to enhance plant resistance to various adverse stresses has received increasing attention. Joner and Leyval (2003) found that the inoculation of AMF could increase the degradation of polycyclic aromatic hydrocarbons in the rhizosphere of *Trifolium repens* L. and *Lolium multiflorum* Lam., and bioconcentration factors of phenanthrene and pyrene tended to decrease with increasing concentrations of phenanthrene and pyrene in the soil. Fungal hyphae emanated from mycorrhizal roots colonized in the petroleum hydrocarbon contaminated soil for over 16 weeks, with mycorrhizal fungi served as carriers (Sarand et al., 1998; Frey-Klett et al., 2007). In another study, Liu et al. (2004) showed that inoculation of AMF could increase the activity of enzymes in soil, thereby enhancing the degradation of benzoapyrene. AMF could also speed up the degradation process of di-(2-ethylhexyl) phthalate in soils, indicating that hyphae played an important role in the degradation and translocation of di-(2-ethylhexy) phthalate (Wang et al., 2002). Petroleum hydrocarbons induced the formation of reactive oxygen species including free radicals such as superoxide, hydrogen peroxide and hydroxyl in plants, and increased the activities of antioxidant enzymes of superoxide dismutase (SOD), catalase (CAT) or peroxidase (POD) (Parida et al., 2004). The combined action of SOD, CAT and POD is critical in mitigating the effects of oxidative stress, since the SOD

* Corresponding author. Tel./fax: +86 029 87080157.
E-mail address: tangm@nwsuaf.edu.cn (M. Tang).

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merely acts on the superoxide anion converting it to another reactive intermediate (H_2O_2) while CAT and POD act on H_2O_2 converting it to water and oxygen (Mate's, 2000). Although much has been reported that AM fungi could improve plants' stress resistance and reduce damage from petroleum hydrocarbons, there is a conspicuous lack of information related to the growth and physiological aspects of the plant, and the metabolic relationships between petroleum hydrocarbons and enzymatic antioxidant levels under diesel stress. For example, how does inoculated AMF affect biomass of *Zea mays* seedlings? What are the effects of inoculated AMF on the relative contents of chlorophyll, soluble proteins, malondialdehyde, free proline and antioxidant enzymes under diesel stress? This study intended to provide answers to these questions based on our laboratory study, and attempts were made to examine the metabolic relationships between petroleum hydrocarbons and enzymatic antioxidant levels and to elucidate the mechanism that enhances the tolerance of diesel stresses.

2. Materials and methods

2.1. Materials

Glomus constrictum was isolated from the rhizosphere soil of *Z. mays* in Moxi oil field where the soil had been contaminated by oil. The single spore of *G. constrictum* was cultivated with *T. repens* for 60 days as the inoculums for *Z. mays*. The inoculums consisted of rhizosphere soil, spores (the spore density was 289–345 spores per 100 g dry soil) and mycelium of *G. constrictum*, and plus the infected *Trifolium* root fragments with an infection level of 95.35% were then inoculated to *Z. mays* that grew for another 60 days.

Z. mays cultivars Yuyu 22 were provided by Shaanxi Academy of Agricultural Sciences. Having been sterilized with 30% hydrogen peroxide for 5 min, the seeds were washed with distilled water and soaked for 2 h. The imbibed seeds were germinated in incubator at 28 °C (Liu and Li, 2000).

The soil used in this study was collected from the top layer (0–20 cm) of a soil in Yangling City, Shaanxi Province, China. The soil had a pH of 7.65 (soil/water ratio of 1:2.5 W/V), an organic matter content of 6.05 g kg^{-1}, an available nitrogen (NO_3–N) of 36.8 mg kg^{-1}, a phosphorus (P_2O_5) of 17.34 mg kg^{-1} and potassium (K_2O) of 82 mg kg^{-1}, measured according to the method described by Bao (2000), was subsequently ground, sieved through a 2 mm sieve before performing chemical analyses, and mixed with fine sand (sand/soil, 1:2 V/V). The mixture was autoclaved at 121 °C for 2 h.

The experimental organic pollutant was diesel #20 (Table 1). The oil sample was first treated at 80 °C for 4 h in an oven to remove volatile hydrocarbons, and then kept in a brown glass bottle.

2.2. Methods

The treatments were factorial combinations of two factors: (1) non-mycorrhizal control and *G. constrictum* as a mycorrhizal inoculum and (2) five oil levels of 0, 2, 6, 10 and 15 g kg^{-1}. The experimental plots, 15 cm × 13 cm in size, were disinfected with 0.1% potassium permanganate solution for 2 h before soil was loaded. Diesel was first dissolved in chloroform and then prepared at five different concentrations. Five soil samples were mixed evenly with different concentrations of diesel, and subsequently placed in a ventilated area for seven days at room temperature to allow the chloroform to volatilize. Each pot was first filled with 1.5 kg prepared soil, then 20 g inoculums were spread over the surface; for the non-mycorrhizae control the inoculums had been autoclaved. Finally the pots were covered with 0.5 kg prepared soil. Each treatment was repeated four times. Four maize seeds were sowed initially and two well-developed seedlings were retained after 10 days. All experiments were carried under 12 h day light per day and 20–35 °C. Each pot was irrigated with 100 ml of Hoagland (Hoagland) nutrient solution 2–3 times a week. The growth and physiological parameters of *Z. mays* were measured after 12 weeks.

Roots were collected, washed gently with tap water, and dried with paper towels, cleared 30 min in 10% KOH at 90 °C, rewashed, acidified in 4% lactic acid and stained in 0.1% trypan blue in lacto-glycerol. The percentage of root length colonized by AMF was measured using a compound microscope as described by Gong et al. (1997). The dependence of mycorrhizae was defined as the percentage of the plant growth that was subject to the adding of AMF, and calculated with the following formula (Menge et al., 1978):

$$\mathrm{MD}(\%) = (\text{mycorrhizal plant dry weight}/\text{non-mycorrhizal plant dry weight}$$

Heights and diameters were scaled with tape and vernier caliper. Plant biomass was weighed, and the relative content of chlorophyll was measured using SPAD-502 portable chlorophyll apparatus. The contents of malondialdehyde (MDA), proline and soluble protein, and the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were determined according to the methods described by Gao (2000).

2.3. Statistical analysis

Experiments were conducted with four replicas per treatment. The data were subject to two-factor ANOVA with diesel and AMF treatments as independent variables; when a significant effect was found, and the means were compared by Duncan's Test at the 5% level (SAS version 8.0).

3. Results

3.1. The effects of AMF on the growth of Z. mays under diesel stress

With the increase of the diesel concentration in the soil, the infection rate of AMF on *Z. mays* roots gradually declined. When the concentration was greater than 6 g kg^{-1}, the infection rate of AMF decreased significantly ($P < 0.05$) (Table 2). The infection rate reached its peak level of 91.50% when the concentration was 2 g kg^{-1}, and dropped to the lowest level of 47.30% at the concentration of 15 g kg^{-1}.

The mycorrhizal plant was taller than the non-mycorrhizal one at the same experimental diesel concentration (Table 5). The percentages of height increase of the AMF-inoculated, compared with the non-inoculated, ranged from 0.08% to 47.20%. The significant differences were observed in those treatments with diesel concentration of 6, 10 and 15 g kg^{-1} (Table 2).

At the concentration of 0, 2, and 6 g kg^{-1} there was no difference observed between the inoculated and the non-inoculated plants, the basal diameter of the mycorrhizal plant was significantly larger

Table 1
The characters of diesel #20.

Index	Diesel #20
$^{O}E_{80}$	≤5.0
Flash point (°C)	≥80
Freezing point (°C)	≤15
Ash content (%)	≤0.3
Water content (%)	≤1.0
Sulphur content (%)	≤1.0
Mechanical impurity (%)	≤1.5