International Biodeterioration & Biodegradation 105 (2015) 58–65

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Is the arbuscular mycorrhizal fungus *Rhizophagus irregularis* able to fulfil its life cycle in the presence of diesel pollution?

Sihem Driai [a, b], Anthony Verdin [a], Frédéric Laruelle [a], Arifa Beddiar [b], Anissa Lounès-Hadj Sahraoui [a, *]

[a] Unité de Chimie Environnementale et Interactions sur le Vivant (UCEIV), Université du Littoral Côte d'Opale (ULCO), 50 Rue Ferdinand Buisson, BP 699, 62228 Calais Cedex, France
[b] Laboratoire de Biologie Végétale et Environnement, Université Badji Mokhtar, BP 12, 23000 Annaba, Algeria

ARTICLE INFO

Article history:
Received 28 May 2015
Received in revised form
14 August 2015
Accepted 17 August 2015
Available online 29 August 2015

Keywords:
Mycorrhiza
Rhizophagus irregularis
Cichorium intybus L.
Diesel
Soil pollution
Oxidative stress

ABSTRACT

The present work examined the impact of increasing diesel concentrations (0.05, 0.1, 0.25, 0.5 and 1%) on the development of both partners of the arbuscular mycorrhizal symbiosis: *Rhizophagus irregularis* and chicory roots (*Cichorium intybus* L.) grown *in vitro*. Our findings showed that although the different diesel concentrations tested (0.05, 0.1, 0.25, 0.5%) affect negatively the main stages of *R. irregularis* development (germination, germinative hyphal elongation, root colonization rate, extraradical hyphae development, sporulation) and the chicory root growth, they are not completely inhibited, except at 1%. The arbuscular mycorrhizal fungus was able to fulfil its life cycle in the presence of the pollutant. No increase in malondialdehyde (MDA) production – a biomarker of lipid peroxidation – was detected in diesel-exposed mycorrhizal or non-mycorrhizal roots, suggesting that the negative effect of diesel on the chicory roots growth could not result from the alteration of membrane lipids. Moreover, our results pointed out that the diesel toxic effect on the growth of chicory roots is less noticeable when they are mycorrhized, indicating a protective effect of mycorrhization. This protection could be related to induction of antioxidant enzyme peroxidase activity, but not to superoxide dismutase activity. Taken together, our results demonstrated the toxic effect of diesel on the mycorrhizal symbiosis and suggest a probable involvement of the mycorrhizal fungus in the protection of chicory roots against oxidative stress induced by diesel.

1. Introduction

Diesel is a hazardous fuel commonly used for vehicles and machinery. Its composition is very varied and dependent on its origin and on current legislation. It is a complex mixture of petroleum hydrocarbons (HC) with an average carbon number of C8–C26. The majority of its components consist of alkanes, both straight chained and branched and aromatic compounds including mono-, di- and polyaromatic hydrocarbons, which are relatively persistent in the soil (Adam and Duncan, 1999). The low molecular weight of diesel HC compounds, make of it more toxic than some of other petroleum products because the low molecular weight compounds are more soluble and bioavailable than high molecular weight ones (Kauppi et al., 2011). The increased use of diesel may cause a permanent risk to the environment and to humans (Abed et al., 2002). The presence of diesel HC in the environment not only adversely affects human health but also plant growth and development. Diesel hydrocarbons hinder the growth of plants, the multiplication of many other microorganisms and the microbiological processes in soil.

The toxic effect of organic pollutants on plants and soil microorganisms has been demonstrated by several authors (Leyval and Binet, 1998; Verdin et al., 2006; Debiane et al., 2008, 2009; Campagnac et al., 2010; Hernández-Ortega et al., 2012). Organic pollutants can limit plant growth and mineral nutrient absorption (Merkl et al., 2005; Hernández-Ortega et al., 2012). They can act directly by inhibiting seed germination and seedling root growth (Adam and Duncan, 1999; Maila and Cloete, 2002) and reducing photosynthetic rates (Macfie and Taylor, 1992), or indirectly by

Abbreviation: AMF, arbuscular mycorrhizal fungi; SOD, superoxide dismutase; POD, peroxidase; MDA, malondialdehyde; ROS, reactive oxygen species.

* Corresponding author.
E-mail addresses: driai_sihem@yahoo.fr (S. Driai), lounes@univ-littoral.fr (A. Lounès-Hadj Sahraoui).

http://dx.doi.org/10.1016/j.ibiod.2015.08.012
0964-8305/

changing soil chemical parameters (Kisic et al., 2009) and affecting soil microorganisms activities (Lapinskiene et al., 2006; Barrutia et al., 2011; Guo et al., 2012). Moreover, organic pollutants induce the formation of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl ($OH^{\bullet}$) (Parida et al., 2004). Among the oxidative damages caused by ROS, there is peroxidation of membrane lipids that can generate malondialdehyde (MDA) used as a biomarker of membrane alterations (Bailly et al., 1996; Arora et al., 2002; Goel and Sheoran, 2003). Plants have the ability to maintain a non-toxic level of ROS using non-enzymatic (Dat et al., 2000) and enzymatic antioxidant systems such as superoxide dismutase (SOD) which catalyses the dismutation of $O_2^{\cdot-}$ to H_2O_2, and peroxidase (POD) which decomposes H_2O_2 (Arora et al., 2002).

To overcome the detrimental effects and to improve plant tolerance to stresses, the plants adopt several strategies including the development of symbiosis with arbuscular mycorrhizal (AM) fungi. Present in the soils of most ecosystems, AM fungi form symbiotic associations with the roots of over 80% of the terrestrial plant species (Smith and Read, 2008). Through its mycelium network, mycorrhizal symbiosis improves plant water and nutrient uptake especially phosphorus (Schreiner, 2007; Mardukhi et al., 2011; Hernández-Ortega et al., 2012; Labidi et al., 2012). Arbuscular mycorrhizal fungi also play a role in increasing plant tolerance to abiotic stresses such as pollutants (Leyval et al., 2002; Alarcón et al., 2006; Verdin et al., 2006; Debiane et al., 2009), and biotic stresses such as plant pathogens (Dalpé, 2005; Akhtar and Siddiqui, 2008). It was also demonstrated that AM fungi can facilitate plant establishment and survival in hydrocarbons-contaminated soils and also contribute to increase hydrocarbon biodegradation in the plant rhizosphere through stimulation of soil microbial communities with xenobiotic degradation abilities (Binet et al., 2000; Zhou et al., 2013; Nwoko, 2014). However, little is known about the impact of organic pollutants such as diesel on AM fungi development.

Thus, this work aims to study, in monoxenic conditions, the impact of increasing diesel concentrations on the main stages of the AM fungus *Rhizophagus irregularis* development (spore germination, germinative hyphae elongation, root colonization rate, extraradical hyphae development and sporulation) and on the growth of its host, chicory roots (elongation and dry biomass). At the biochemical level, oxidative stress biomarkers such as the production of MDA as well as SOD and POD enzyme activities are assessed.

2. Material and methods

2.1. Plant and fungal material

R. irregularis MUCL 43194 (DAOM 197198) (Schüßler and Walker, 2010) spores were used in the first experiment (spore germination kinetics). Chicory root cultures (*Cichorium intybus* L.), transformed with *Agrobacterium rhizogenes* and colonized or not by the same AM fungus *R. irregularis*, were undertaken in the second experiment (*in vitro* culture of mycorrhizal and non-mycorrhizal chicory roots).

2.2. Preparation of the culture medium

The culture medium used is the modified M medium (Bécard and Fortin, 1988) solidified with 0.3% (w v-1) gellan gel (Phytagel; Sigma, St. Louis, MO, USA) and autoclaved at 120 °C for 20 min. The diesel was filter sterilized at 0.25 μm in a horizontal flow hood and added to the modified M medium in order to obtain the concentrations of 0.05, 0.1, 0.25, 0.5 and 1%. The control used in this experiment is the M medium without diesel (0% diesel). The diesel has a cetane number of 51 and a total polycyclic aromatic hydrocarbons amount less or equal to 8.0% (w/w).

Addition of diesel at the concentration of 0.05% did not change the pH value of the M medium which was equal to 5.9. A slight decrease of 0.01 and 0.02 unit in the pH values was measured at the 0.1 and 0.25% diesel concentrations respectively when compared with the control. At 0.5 and 1% diesel concentrations, the pH culture medium values decreased from 5.9 to 5.63 and 5.42 respectively.

2.3. Methods

2.3.1. Germination of R. irregularis spore experiment

Spores were extracted from the fungal compartment of a 2-month-old monoxenic culture of Ri T-DNA transformed chicory (*C. intybus* L.) roots colonized by *R. irregularis*. One spore was placed in the middle of each Petri dish (5.5 cm diameter) containing 10 mL of the M medium supplemented or not (Control) with the different diesel concentrations (0.1, 0.25, 0.5 and 1%). The dishes were incubated for 30 days at 27 °C in the dark.

2.3.1.1. Determination of R. irregularis spore germination kinetics and germination hypha development. The number of germinated spores was counted under an optical microscope (GX100) after 3, 5, 7, 10, 20 and 30 days of incubation. A spore is considered as germinated when a germ tube is observed. After 30 days of incubation, the hyphal length was measured under an optical microscope using the method of Newman (1966), and the number of spores germinating in a branched or linear mode was counted.

2.3.1.2. Spore viability test. Non-germinated spores were collected and then rinsed with sterile distilled water and transferred onto a diesel free medium. The spore cultures were incubated during one week at 27 °C in the dark. The germinated spores were counted under an optical microscope (GX100).

2.3.2. In vitro culture of chicory root colonized or not with R. irregularis experiment

Mycorrhizal and non-mycorrhizal chicory roots transformed by *A. rhizogenes* (Fontaine et al., 2004) were grown in a standard mono-compartmental Petri dishes (9 cm diameter) containing 25 mL aliquot of the modified M medium supplemented or not (control) with increasing diesel concentrations (0.05, 0.1, 0.25, 0.5 and 1%). A piece of agar (1.5 cm^2;) of 2-month-old mycorrhizal or non-mycorrhizal chicory root was placed on the middle of each Petri dish. Five dishes per treatment were prepared and incubated in an inverted position during 9 weeks at 27 °C in the dark.

2.3.2.1. Determination of fungal and root growth. After 9 weeks of incubation, the hyphal lengths were measured using the method described by Declerck et al. (2003) which consists in counting the number of intersections of the roots on a circular gridlines and applying the formula of Newman (1966). Root and extraradical hyphal lengths were determined using the same methods described for the determination of hyphal lengths. The number of spores formed was determined by adding the number of spores recorded in each cell of the circular gridlines (Declerck et al., 2001). The roots were collected after solubilising the culture media during 15 min under agitation in 25 mL of Tris–HCl buffer (50 mM, pH 7.5) + EDTA (10 mM) (v/v), and collected by filtration on a 0.5 mm sieve. Roots were then rinsed with distilled water. A first aliquot of the mycorrhizal roots collected from each replicate were cleared in a solution of KOH 10% (w v-1) and stained with 0.05% blue trypan in lactic acid as described by Phillips and Hayman (1970) to determine arbuscular mycorrhizal colonization using the gridline intersect