BRAZILIAN JOURNAL OF MICROBIOLOGY xxx (2017) xxx–xxx

BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Environmental Microbiology

Arbuscular mycorrhizal fungal communities in the rhizosphere of a continuous cropping soybean system at the seedling stage

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ARTICLE INFO

Article history:
Received 20 September 2016
Accepted 1 March 2017
Available online xxx

Associate Editor: Jerri Zilli.

Keywords:
Soybean
Continuous cropping
Arbuscular mycorrhizae
PCR-DGGE
Genetic diversity

ABSTRACT

Arbuscular mycorrhizae (AM) fungi play a crucial role in the growth of soybean; however, the planting system employed is thought to have an effect on AM fungal communities in the rhizosphere. This study was performed to explore the influence of continuous soybean cropping on the diversity of Arbuscular mycorrhizal (AM) fungi, and to identify the dominant AM fungus during the seedling stage. Three soybean cultivars were planted under two and three years continuous cropping, respectively. The diversity of AM fungi in the rhizosphere soil at the seedling stage was subsequently analyzed using polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE). The results showed that an increase in cropping years improved the colonization rate of AM in all three soybean cultivars. Moreover, the dominant species were found to be *Funneliformis mosseae* and *Glomus* species. The results of cluster analysis further confirmed that the number of years of continuous cropping significantly affected the composition of rhizospheric AM fungal communities in different soybean cultivars.

Introduction

Soybean is the fourth most important grain cultivar in the world, and an important oil crop in China. Soybean contains active substances that are beneficial to human health such as soy proteins, isoflavones, saponins and oligosaccharides.[1]

Considerable attention has recently been directed toward the increasingly severe problem of root rot in soybean under intensified cultivation. Continuous cropping has resulted in an increase in diseases and pests, poor soil chemical and physical properties, a drastic reduction in yield and, in some cases, total crop failure.[2–4] Tackling the yield reductions resulting from continuous cropping has therefore become a key

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https://doi.org/10.1016/j.bjm.2017.03.017

Please cite this article in press as: Cui J, et al. Arbuscular mycorrhizal fungal communities in the rhizosphere of a continuous cropping soybean system at the seedling stage. *Braz J Microbiol.* (2017), https://doi.org/10.1016/j.bjm.2017.03.017

issue in the soybean industry. Recent studies suggest that continuous cropping of soybean leads to drastic changes in the rhizosphere microflora, with a gradual transition from bacterial-dominant highly fertile soil to fungal-dominant low fertile soil, and therefore, microbiota dominated by fungi.[5] Moreover, the concentration of root exudates such as phenolic acids is significantly correlated with fungal quantity, while increased levels of soil organic compounds (sugars, amino acids and organic acids) as a result of continuous cropping play a facilitative role in the growth of root rot pathogens. Since soil sterilization treatments have no effect on fungal spores, a gradual transition to a dominant fungal community is subsequently observed.[6]

Arbuscular mycorrhizae (AM) fungi are the most important type of endotrophic mycorrhiza, and are associated with the majority of terrestrial plants. AM fungi are a group of ubiquitous microbes in nature, and are one of the major types in the rhizosphere,[7–10] significantly improving the absorption and utilization of nutrients by host plants. AM fungus also stimulate growth, increase stress and disease resistance, and promote community succession and ecosystem stability.[11] AM fungi are known for several effects and can be used as bio-fertilizer.[12–16] Application of AM fungi is widely recognized as important for many crops including soybean.[17,18] AM fungi applied to soybean can improve the absorption of nutrients in the host plants, improve the nitrogen fixation capability in *Rhizobia* and also the colony structure of the rhizospheric econiche, thus increasing the yield and economic benefits of soybean.[19,20] The antagonistic effects of AM fungus on the problems associated with continuous cropping have therefore been examined.[21,22] However, the diversity of AM fungi in rhizospheric soil with different soybean cultivars under different continuous cropping regimes remains largely unknown.

In this study, three typical soybean cultivars (Heinong 37, Heinong 44 and Heinong 48) grown widely in Heilongjiang province, China, were selected to examine the diversity of AM fungi in the rhizosphere during continuous cropping. Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was employed with the aim of identifying dominant AM Fungi communities. The findings provide a theoretical basis for further studies of the relationship between AM fungi and soil-borne pathogens.

Materials and methods

Experimental plots

This study was carried out at the Experimental Station of the Research Institute of Sugar Industry, Harbin Institute of Technology, China (125°42′–130°10′E, 44°04′–46°40′N). Soybean cultivars were planted in the same plot and subjected to two or three years continuous cropping, respectively. Management techniques in the trial field were the same as those in standard production fields. Organic matter, potassium, phosphorus and nitrogen contents of the soil were determined using the Ignition method, NaOH melt-flame photometry method, alkali fusion-Mo-Sb Anti spectrophotometric method and Kjeldahl method, respectively.

Soybean cultivars

Three typical soybean cultivars planted widely in Heilongjiang Province were used: 'Heinong 44' (high oil, 36% average protein content, 23% average fat content, designated HN44), 'Heinong 37' (intermediate cultivar, 40% average protein content, 20% average fat content, designated HN37), and 'Heinong 48' (high protein, 45% average protein content, 19% average fat content, designated HN48). Each cultivar was subjected to two and three years continuous cropping, respectively, as follows: 2 years continuous cropping of HN37 (designated C2HN37), 2 years continuous cropping of HN44 (C2HN44), 2 years continuous cropping of HN48 (C2HN48), 3 years continuous cropping of HN37 (C3HN37), 3 years continuous cropping of HN44 (C3HN44) and 3 years continuous cropping of HN48 (C3HN48). The protein content of the soybean seeds was determined using the Kjeldahl method and the fat content determined using the Soxhlet extraction method.

Harvesting and processing of samples

Soybean samples were collected after 30 d (at the seedling stage) along with soil samples. Three soybean plants per cultivar were randomly selected from each treatment by digging to a depth of 10–20 cm. The plants were shaken gently and any soil adhering to the root surface 1–3 mm removed using a brush as rhizosphere soil. The three soil samples per treatment were subsequently mixed as a collective sample. Samples were then air dried then stored at 4 °C until use. The roots were then washed for soil sample collection, and three randomly selected segments removed with scissors and used as a collective sample. All treatments were sampled in triplicate. Roots were then washed in distilled water and placed in FAA fixative and the level of AM fungal colonization determined microscopically. Roots were stained using alkali hydrolyzable and acid fuchsin, then stored at 4 °C until use. Root and soil samples from each cropping system were denoted RL2 and RL3, and SL2 and SL3 to represent 2 and 3 years continuous cropping, respectively.

Determination of the rate of AM fungal colonization

Fifty fibrous root segments from each root sample were selected randomly, stained, prepared on slides and examined microscopically to observe the level of AM colonization. Colonization rates of each fibrous root segment were assigned values of 0, 10, 20, 30, 40 and 100% based on the microscopic observations and the colonization frequency (*F*) of each cultivar under each cropping system calculated as follows:

$$F\,(\%) = \text{number of colonized root segments/total number of root segments.}$$

AM colonization intensity (*M*) was then calculated statistically for each fibrous root segment as follows[23]:

$$M\,(\%) = (0\% \times \text{number of root segments} + 10\% \times \text{number of root segments} + 20\% \times \text{number of root segments} + \ldots 100\% \times$$

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