European Journal of Soil Biology 75 (2016) 1–7

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Effects of seaweed fertilizer on the growth of *Malus hupehensis* Rehd. seedlings, soil enzyme activities and fungal communities under replant condition

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ARTICLE INFO

Article history:
Received 14 June 2015
Received in revised form
2 April 2016
Accepted 5 April 2016
Available online 19 April 2016

Handling Editor: C.C. Tebbe

Keywords:
Seaweed fertilizer
Malus hupehensis Rehd.
Antioxidant enzyme activities
Soil enzyme activities
Soil fungal communities
Terminal restriction fragment length polymorphism

ABSTRACT

Seaweed and its derivatives are widely used as nutrient supplements, biofertilizers, and biostimulants for soil in agriculture. The aim of this study was to evaluate the effects of seaweed fertilizer on the growth of apple (*Malus hupehensis* Rehd.) seedlings under replant conditions. We investigated the growth of apple seedlings in replant soil treated with seaweed fertilizer at application rates of 0, 5, 20, and 40 g kg^{-1}. The addition of seaweed fertilizer significantly increased the plant height and dry weight of apple seedlings. Seedlings grown in soil treated with seaweed fertilizer, particularly the dose of 40 g kg^{-1} soil showed higher activities of antioxidant enzymes including superoxide dismutase, peroxidase and catalase, which was accompanied by lower malondialdehyde accumulation. The activities of soil enzymes (invertase, urease, proteinase and phosphatase) were higher in soil treated with seaweed fertilizer than in control soil. An analysis of terminal restriction fragment length polymorphism profiles showed that the fungal communities differed markedly between the 40 g kg^{-1} seaweed fertilizer treatments and the 0, 5, and 20 g kg^{-1} treatments. The highest values of Shannon diversity index, evenness index and richness index were in the 40 g kg^{-1} seaweed fertilizer treatments, and the lowest values of these indexes were in the control. These results suggested that seaweed fertilizer application at a dose of 40 g kg^{-1} can improve soil enzymes activities, change the soil fungal communities, and improve soil quality. These changes can promote seedlings growth, increase antioxidant activity, and decrease lipid peroxidation in roots, thereby alleviating apple replant disease.

1. Introduction

Apple is one of the most important fruits trees worldwide, and China has the largest area of apple tree cultivation (FAOSTAT, 2010). Apple replant disease (ARD) is widespread in China because repeated production in the same field is a common practice owing land scarcity and the replant of aged apple orchards. This disease appears commonly in all the major apple-growing regions around the world [1]. The causes of ARD are diverse and complex, but accumulated evidence indicates that the causes of ARD are a consortium of biological agents including nematodes, oomycetes bacteria, and fungal species [1–3]. For example, *Pratylenchus penetrans* Cobb is considered to be the main nematode species involved in ARD [3]. Several *Rhizoctonia* species, including the multinucleate *Rhizoctonia solani* Kühn AG-5 and AG-6, showed high virulence to apple in several studies [4,5]. *Fusarium* isolates, such as *Fusarium tricinctum* (Corda) Sacc, *Fusarium solani* (Mart.) Sacc. and *Fusarium avenaceum* (Fr.) Sacc. have also been found to be pathogenic to apple [2,4].

The effectiveness of crop rotation is limited once ARD occurs because the causal pathogens are persistent in soil [5]. Large amounts of chemical fungicides are often applied to overcome the problem of ARD. However, these chemical fungicides are harmful to the environment. Thus, it is important to develop a better ARD control method to ensure a healthy apple industry without

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http://dx.doi.org/10.1016/j.ejsobi.2016.04.003
1164-5563/

negative effects on farmers' incomes.

Soil amendments could be a promising method to control soil disease including ARD. Soil amendments range from bio-control fertilizer to organic matter [6,7]. Bioorganic fertilizer has been shown to inhibit the growth of soilborne fungal pathogens [6]. Similarly, organic amendments such as brassicaceae seed meal and organic matter compost have been shown to modify the soil microbial community such that the growth of disease-causing organisms is suppressed, resulting in conditions that are favorable for plant growth [7,8].

Seaweeds are important marine living resources with tremendous commercial application, and a number of commercial products from seaweed extract are used in agriculture and horticulture. Seaweed extracts can be used in liquid form as a foliar spray, or soil drench, or in powder and granular forms as soil conditioners and manure [9–11]. Using these extracts as fertilizers can potentially improve plant productivity because they contain growth-promoting hormones [9]. Several studies have also shown that seaweed extracts can suppress diseases and insect pests [9,12].

To date, there have been few studies on the effects of seaweed fertilizers on soil attributes (enzyme activities and fungal community diversity) under replant conditions. Additionally, little is known about the effects of seaweed fertilizer on the activities of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), which are important for eliminating excessive reactive oxygen species (ROS) from plant tissues [13]. Therefore, the objectives of this study were to investigate: (I) the effects of seaweed fertilizer on plant growth and on the antioxidant system in roots of apple seedlings under replant conditions; and (II) the effects of seaweed fertilizer on soil enzyme activities and the soil fungal community. The fungal community was evaluated by terminal restriction fragment length polymorphism (T-RFLP) analyses. The overall aims of this research were to understand the effect of seaweed fertilizer on the tolerance of apple seedlings to ARD and to explore a solution for alleviating ARD.

2. Materials and methods

2.1. Experimental materials

The *Malus hupehensis* Rehd. seedlings were used in this work. Seeds were stratified at 4 °C to be germinated for 30 days, and then sown in nursery plates. Uniform seedlings were planted into pots when the seedlings reached six leaves. The pots contained soils with different doses of seaweed fertilizer.

The seaweed fertilizer used in this study was produced from *Lessonia nigrescens* and *Lessonia flavicans* by the Qingdao Bright Moon Seaweed Group Co. Ltd. (Qingdao, China). The seaweed was soaked in water (15 water: 1 seaweed), thoroughly washed, cut into small pieces, crushed, boiled in a high-pressure reaction pot, and then filtered. Finally, a carrier was added to the filtrate and the mixture was dried to obtain the seaweed fertilizer. The dried fertilizer was sieved through a 2 mm mesh sieve and its chemical and physical characteristics were analyzed (Table 1).

Table 1
Chemical and physical characteristics of the replant soil and seaweed fertilizer applied.

	Replant soil	Seaweed fertilizer
Organic matter (g kg^{-1})	21.2	301.4
NO_3–N (mg kg^{-1})	78.6	1203
NH_4–N (mg kg^{-1})	5.1	35.2
Available P (mg kg^{-1})	78.6	2387
Available K (mg kg^{-1})	60.2	4256

Soil used for the experiment was collected from a 50-year-old apple orchard located in Taian, Shandong Province, China. The replant soil was collected as described previously [14]. The soil was sandy loam and its characteristics are shown in Table 1. The results of standard ARD bioassays predicted the appearance of ARD in apple seedlings grown in this soil [3].

2.2. Experimental design

The seaweed fertilizer was mixed well with replant soil at the concentrations of 0, 5, 20, and 40 g kg^{-1}, and then 6.0 kg of each soil was added to each pot (outer diameter, 27 cm; inner diameter, 23 cm; height, 18 cm). Uniform apple seedlings of *Malus hupehensis* Rehd. were transplanted into the pots on May 1, 2013. Two seedlings were planted in each pot, and 30 pots were prepared for each treatment. The pots were arranged in a completely randomized design. Water and nutrients were managed according to usual practice, and the N, P, and K concentrations were normalized among the various treatment.

2.3. Sampling

The first samples were collected on July 20 when there were visible differences in seedlings growth among the treatments. The remaining samples were harvested on August 20 and September 20, respectively. The root samples were frozen in liquid nitrogen, and then stored at −20 °C until analysis. At the same time, soil samples were collected and divided into two portions. The first portion was air-dried to determine soil enzyme activities, and the second portion was stored at −20 °C for the T-RFLP analysis. Plant height and dry weight, antioxidant enzymes activities in the root, and soil enzyme activities were determined in July, August and September, while the T-RFLP analyses were only conducted for samplings collected in September.

2.4. Determination of antioxidant enzymes and malondialdehyde in seedlings root

Antioxidant enzymes (CAT, POD and SOD) were extracted and their activities quantified as described elsewhere [15]. One unit (U) of SOD activity was defined as the amount of enzyme causing 50% of the maximum inhibition of the reduction of nitroblue tetrazolium (NBT), and SOD activity is expressed as U g^{-1} FW. One unit (U) of POD and CAT activity was defined as the amount of enzyme causing a change of 0.1 in the absorbance at 470 and 240 nm per minute, respectively. and the activity of POD and CAT are expressed as U g^{-1} FW min^{-1}.

Lipid peroxidation in the roots was measured by detecting malondialdehyde (MDA) accumulation [15]. The concentration of MDA was determined by the extinction coefficient of 155 $mmol^{-1}$ cm^{-1}, and is expressed as mmol g^{-1} FW.

2.5. Determination of the soil enzyme activities

The soil urease, invertase, and neutral phosphatase activities were determined according to the method of Guan [16]. Soil proteinase activity was determined according to Ladd and Butler [17]. The activities of soil invertase, urease, proteinase and phosphatase are expressed as mg glucose, NH_3–N, NH_2–N, and phenol released per 1 g dry soil per 24 h, respectively.

2.6. Terminal restriction fragment length polymorphism analysis

The DNA was extracted from 0.5 g soil using an E.Z.N.A.™ Soil DNA Kit (Omega Bio-tek. Omc. USA) according to the