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Piriformospora indica confers drought tolerance on *Zea mays* L. through enhancement of antioxidant activity and expression of drought-related genes

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ABSTRACT

Drought stress is one of the most severe environmental constraints to plant growth and crop productivity. Plant growth is greatly affected by drought stress, and plants, to survive, adapt to this stress by invoking different pathways. *Piriformospora indica*, a root-colonizing endophytic fungus of Sebacinales, promotes plant growth and confers resistance to biotic and abiotic stresses, including drought stress, by affecting the physiological properties of the host plant. The fungus strongly colonizes the roots of maize (*Zea mays* L.) and promotes shoot and root growth under both normal growth conditions and drought stress. We used polyethylene glycol (PEG-6000) to mimic drought stress and found that root fresh and dry weight, leaf area, SPAD value, and leaf number were increased in *P. indica*-colonized plants. The antioxidative activities of catalases and superoxide dismutases were upregulated within 24 h in the leaves of *P. indica*-colonized plants. Drought-related genes *DREB2A*, *CBL1*, *ANAC072*, and *RD29A* were upregulated in drought-stressed leaves of *P. indica*-colonized plants. Furthermore, after drought treatment, proline content increased, whereas accumulation of malondialdehyde (MDA), an indicator of membrane damage, decreased in *P. indica*-colonized maize. We conclude that *P. indica*-mediated plant protection against the detrimental effects of drought may result from enhanced antioxidant enzyme activity, proline accumulation, and expression of drought-related genes and lower membrane damage in maize plants.

1. Introduction

Drought is one of the most severe abiotic stresses constraining and destabilizing maize grain production [1], and the sensitivity of this crop to water stress severely reduces its yield [2]. *Piriformospora indica*, a basidiomycete of the Sebacinaceae family, was first isolated from bush rhizosphere zones of the Thar Desert in India [3]. *P. indica*, which is easily cultivated in axenic culture, is an endophytic fungus that colonizes the roots of a broad range of hosts, promoting host plant growth and

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increasing plant tolerance to biotic and abiotic stresses by affecting physiological properties [3–5]. Drought can induce the production of reactive oxygen species (ROS), which cause damage to lipids, carbohydrates, proteins, and DNA [6–9]. Plants possess a collection of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) that alleviate oxidative stress [10].

P. indica confers drought tolerance on Chinese cabbage leaves by stimulating antioxidant enzymes and expression of drought-related genes [11]. The fungus also confers drought tolerance on *Arabidopsis* in a manner that is associated with priming of the expression of a set of stress-related genes in leaves [12]. However, it remains unclear whether *P. indica* confers drought tolerance on maize and whether the mechanisms are similar to those in Chinese cabbage and *Arabidopsis*.

The goal of this study was to determine the effect of *P. indica* inoculation on maize tolerance to drought induced by PEG-6000 treatment. Maize inoculated with *P. indica* showed increases in fresh and dry weight, irrespective of the application of drought stress. It is concluded that the mechanisms by which *P. indica* protects maize plants from the detrimental effects of drought may enhance antioxidant enzyme activity and proline accumulation, lower membrane damage, and stronger expression of drought-related genes.

2. Materials and methods

2.1. Plant cultivation

Maize seeds (Jixiang 1) were surface-sterilized with 75% ethanol for 2 min and 0.75% NaClO for 10 min and then washed approximately 5 times with sterilized water, as described by Varma et al. [5]. The seeds were germinated on double-layer filter paper in the dark at 25 °C for 3 days. Young seedlings were inoculated or not with *P. indica* and transplanted to sterilized sand. Twelve plants (used mainly for antioxidant measurement or gene expression analysis) were grown in a single plastic pot (18.5 cm diameter; 13.5 cm height); four plants (mainly for phenotypic analysis) were grown in a square plastic pot (6.0 cm × 6.0 cm; 8.5 cm height, 3 pots used for one treatment). The plants were grown in a greenhouse under 700 μmol m^{-2} s^{-1} light intensity and at temperatures between 25 °C and 28 °C, as described by Ghanem [13].

2.2. Inoculation and root colonization with *P. indica*

Samples of *P. indica* were provided by Dr. Ralf Oelmüller (University of Jena, Germany). The fungus was cultivated in a 250 mL flask filled with *Aspergillus* (ASP) medium. This flask was then placed on a shaker (150 r min^{-1}) and grown in the dark at a constant temperature of 25 °C. After 14 days, the liquid was filtered and the excess culture medium was carefully removed from the mycelia. The maize seedlings germinated on filter paper were inoculated with 10 mL of a 0.1% *P. indica* suspension after being transferred to sand (1 g fresh mycelium per liter of water, injection of the suspension into the sand), and dead mycelia were added into the sand as a control. Root colonization by *P. indica* was examined one week after inoculation. Root systems were harvested from each plant per replication plot and stained with trypan blue. Colonization of roots was assessed using a Leica microscope (DM5000 B; Germany).

2.3. Drought and PEG-6000 treatment

Only one genotype (Jixiang 1) was used for all experiments. For natural drought treatment, 12 plants (per treatment) were grown in a plastic pot containing sterilized sand, and water was withheld. For PEG-6000 treatment, seedlings were carefully removed from sand, washed, and transplanted into a pot with 1/2 strength Hoagland solution containing 20% PEG-6000 (with or without *P. indica* inoculation). Control plants were grown in 1/2 strength Hoagland solution for the duration of the experiment. Samples were harvested within 24 h. All plants were grown in a greenhouse (28 °C/16 h light, 25 °C/8 h dark, light intensity 700 μmol m^{-2} s^{-1}, relative humidity 60%). The experiments were replicated 4 times.

2.4. Vegetative characteristics

After PEG-6000 treatment, root fresh weight, root dry weight, length of the longest root, leaf number, and leaf area of 12 plants per treatment were measured using a balance or a ruler. The SPAD index with or without *P. indica* inoculation was measured using a SPAD-502 Plus (Japan).

2.5. Antioxidative enzyme activity

Antioxidative enzyme activity was determined as described in a previous study [14]. In brief, 0.2 g leaves (fresh weight) was ground and the powder was transferred to tubes containing pre-chilled 50 mmol L^{-1} phosphate buffer (pH 7.8, containing 1% PVP) and centrifuged at 4 °C, 10,000 r min^{-1} for 20 min. Then, 5 mL of the supernatant was transferred to new tubes for SOD, CAT, and POD activity assays according to Giannopolitis and Ries [15], Kraus and Fletcher [16], and Cakmak and Marschner [17], respectively. Five replicates were used for each independent experiment.

2.6. MDA and proline measurements

MDA levels were determined according to the thiobarbituric acid (TBA) method of Hodges [18]. The components in the supernatant of the extract were precipitated with 0.5% TBA. The suspension was then boiled for 10 min and immediately cooled on ice. After centrifugation at 8000×*g* for 10 min, the MDA concentrations of the samples were quantified by measuring absorbance at 532, 600, and 450 nm using a Spectrostar Nano Spectrometer (UV-5800PC, Yuanxi, Shanghai, China).

Proline measurements were performed as previously described by Ashraf and Foolad [19]. Maize leaves were harvested, weighed (approximately 0.2 g fresh weight), and ground to powder in liquid nitrogen; 1 mL 75% ethanol was added, and the sample was shaken overnight. The mixture was centrifuged at 20,000×g and aliquots of the extract were used for measurements. For proline measurement, 100 μL of the aliquot was incubated with 900 μL ninhydrin reagent (1% ninhydrin (*w*/*v*), 60% glacial acetic acid (*v*/*v*), and 40% H_2O) at 100 °C for 1 h. Then, 3 mL toluene was added, followed by