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Dark septate endophyte decreases stress on rice plants

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ABSTRACT

Abiotic stress is one of the major limiting factors for plant development and productivity, which makes it important to identify microorganisms capable of increasing plant tolerance to stress. Dark septate endophytes (DSEs) can be symbionts of plants. In the present study, we evaluated the ability of DSE isolates to reduce the effects of water stress in the rice varieties Nipponbare and Piauí. The experiments were performed under gnotobiotic conditions, and the water stress was induced with PEG. Four DSEs were isolated from the roots of wild rice (*Oryza glumaepatula*) collected from the Brazilian Amazon. Plant height as well as shoot and root fresh and dry matter were measured. Leaf protein concentrations and antioxidant enzyme activity were also estimated. The DSEs were grown *in vitro* in Petri dishes containing culture medium; they exhibited different levels of tolerance to salinity and water stress. The two rice varieties tested responded differently to inoculation with DSE. Endophytes promoted rice plant growth both in the presence and in the absence of a water deficit. Decreased oxidative stress in plants in response to inoculation was observed in nearly all inoculated treatments, as indicated by the decrease in antioxidant enzyme activity. DSE fungi were shown to increase the tolerance of rice plants to stress caused by water deficiency.

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Introduction

Q3 Rice is one of the most consumed cereals in the world due to its nutritional value and because it represents an affordable source of protein.[1] Rice belongs to the genus *Oryza*, which comprises two cultivated species (*Oryza glaberrima* and *Oryza sativa* L.) and a great diversity of wild species (not cultivated).[2]

Water deficiency is one of the most important causes of abiotic stress and compromises global food production, including the production of rice. This stress causes irreversible oxidative damage because the activity of the plant's antioxidant system is not sufficient to limit the reactive oxygen species (ROSs) derived from byproducts of aerobic and photosynthetic metabolism to non-toxic levels. ROSs therefore accumulate in the plant tissues,[3] where they can cause

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damage to cell organelles; oxidize important biological molecules such as nucleic acids, lipids and proteins; and compromise the integrity of the cell membrane and decrease photosynthesis.[4]

Plants respond to these stresses by producing catalase (CAT) and ascorbate peroxidases (APX), the primary enzymes responsible for the maintenance of H_2O_2 at non-toxic levels in cell compartments.[5] However, in a scenario of prolonged stress, the antioxidant system cannot prevent cell damage caused by oxidation, and other mechanisms become relevant. Positive effects of plant associations with beneficial microorganisms under stress conditions have been reported.[6–8]

Dark septate endophytes (DSEs) are conidial or sterile ascomycetous fungi that colonize living plant roots without causing any apparent negative effects.[9,10] They are characterized by intense dark pigmentation and the formation of septate hyphae and occasionally microsclerotia, as well as various arbuscular mycorrhizal fungi (AMF). These fungi can be grown in culture medium and can colonize several plant species. They can be found in plant cortical cells inter- and intracellularly and are present in several environments, even under drought conditions, in the presence of heavy metals and in oligotrophic soils.[11–16] The hypotheses presented thus far for the dominance of DSEs in stressed environments and their effects on host plant protection are primarily related to the presence of the pigment melanin at the endophytes' hyphae and microsclerotia.[12] Melanin can act as an antioxidant agent and also bind heavy metal ions, thereby protecting cell structures from the oxidative damage produced under such conditions.[17] However, the DSE action mechanisms involved in plant protection have not yet been elucidated,[18] but likely involve the presence of extraradical hyphae and extracellular enzymes that can improve soil exploration by roots.[13]

The goals of the present study were to assess the ability of DSE fungi, originating from tropical soils, to grow under stress conditions and to induce stress tolerance in rice plants (*O. sativa* L.).

Material and methods

DSE isolates A, B, C and D (ERR 01, ERR 04, ERR 16 and ERR 46, respectively) obtained from the roots of wild rice (*Oryza glumaepatula*) in the Brazilian Amazon by Ribeiro et al.[19] were tested. These isolates are stored at COFMEA (Embrapa Agrobiology Culture Collection of Micorrhizal Fungi), and they were only partially taxonomically defined up to now.[19] These isolates were previously characterized through amplification and sequencing of the internal transcribed space (ITS1-5S-ITS2), and it was possible to position the isolates at the order level.[19] Following this analysis, A101 is a member of Calosphaeriales, A102 is member of Capnodiales and A103 and A106 are members of Pleosporales. The ITS1-5S-ITS2 sequences are also deposited at the NCBI GenBank, accession numbers KR817246, KR817247, KR817248 and KT780724.[19]

Nipponbare, an improved variety commonly used in rice studies, and Piauí, a wild variety grown in a dry land system, were used in all assays involving plant–fungus interactions.

The capacity of the DSE isolates to grow under stress conditions was tested in two preliminary tests in Petri dishes. Both were placed in a phytotron, using growth medium with sodium chloride (NaCl) or polyethylene glycol (PEG 6000) for the induction of salt and water stress, respectively. The PEG 6000 is a widely used polymer to simulate the effect of drought in studies involving organisms, primarily because it is chemically inert and non-toxic.[20]

For salt stress, 0.2, 0.4, 0.7 or 1 mol L^{-1} NaCl was added to the culture medium at 26 °C to obtain an osmotic potential of −0.49, −0.99, −1.73, and −2.43 MPa, respectively. A control treatment was included with no salt addition. The salt concentrations needed to obtain the different osmotic potentials were calculated using the Van't Hoff equation[21]:

$$\psi_{os} = -RTC,$$

where, ψ_{os} = osmotic potential (atm); R = ideal gas constant (0.082 atm. 1 mol^{-10} K^{-1}); T = temperature (°K); C = concentration (mol L^{-1}).

Fungal mycelial discs 7 mm in diameter, grown for two weeks in potato-dextrose-agar (PDA) growth medium, were placed in Petri dishes containing 39 g L^{-1} commercial PDA medium (Sigma–Aldrich, St. Louis, MO, USA) with the addition of 0, 0.2, 0.4, 0.7 or 1 mol L^{-1} NaCl. Three replicates of each treatment were performed. The dishes were grown for 9 days at 26 °C. Colony diameter was measured after 9 days using a caliper and expressed in mm.

Stress due to PEG addition was measured by placing fungal mycelial discs, also 7 mm in diameter, in Petri dishes containing Hoagland solution solidified with Phytagel (2.5 g L^{-1}), to which PEG-6000 had been added at different concentrations (0, 79.791, 121.139, 180.231 and 264.246 g L^{-1}) to obtain 0, −0.1, −0.2, −0.4, and −0.8 MPa water resistances, respectively.[20] The incubation conditions were the same as the above.

Growth promotion of rice plants by DSE under water deficit

The experiment was maintained in the phytotron in a randomized complete block design in a factorial arrangement 5 × 2 × 5 (four inoculated fungi and an uninoculated control), two rice varieties (Nipponbare and Piauí), and 5 PEG concentrations (0, 79.791, 121.139, 180.231 and 264.246 g L^{-1}), with four replicates (two plants = one experimental unit). The rice plants were grown in Hoagland solution solidified with 2.5 g L^{-1} Phytagel at a mean temperature of 26 °C.

The seeds of the two tested rice varieties were sterilized with 2.5% sodium hypochloride and 70% ethanol for 3 min and inoculated with fungal mycelia grown for two weeks in PDA, followed by pre-germination for 5 days in Petri dishes containing Hoagland solution solidified with 1% agar. Fungal mycelial discs 7 mm in diameter were placed on the dishes' surface close to the seedlings. The control treatments consisted of non-inoculated seeds without the addition of fungal mycelial discs to the growth medium with one PDA disc added (7 mm in diameter).

Following seed pre-germination, the seedlings were transferred into pots containing sterile half-strength Hoagland solution supplemented with 0.3 g L^{-1} $MgSO_4$ and solidified with 2.5 g L^{-1} Phytagel.[22] The pots were kept under a 12 h

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