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Research article

Effect of the fungus *Piriformospora indica* on physiological characteristics and root morphology of wheat under combined drought and mechanical stresses

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ABSTRACT

This study was done to evaluate the effects of the root-colonizing endophytic fungus *Piriformospora indica* on wheat growth under combined drought and mechanical stresses. Inoculated (colonized) and non-inoculated (uncolonized) wheat (*Triticum aestivum* L. cv. Chamran) seedlings were planted in growth chambers filled with moist sand (at a matric suction of 20 hPa). Slight, moderate and severe mechanical stresses (i.e., penetration resistance, Q_p, of 1.17, 4.17 and 5.96 MPa, respectively) were produced by a dead-load technique (i.e., placing a weight on the sand surface) in the root medium. Slight, moderate and severe drought stresses were induced using PEG 6000 solutions with osmotic potentials of 0, −0.3 and −0.5 MPa, respectively. After 30 days, plant physiological characteristics and root morphology were measured. An increase in Q_p from 1.17 to 5.96 MPa led to greater leaf proline concentration and root diameter, and lower relative water content (RWC), leaf water potential (LWP), chlorophyll contents and root volume. Moreover, severe drought stress decreased root and shoot fresh weights, root volume, leaf area, RWC, LWP and chlorophyll content compared to control. Catalase (CAT) and ascorbate peroxidase (APX) activities under severe drought stress were about 1.5 and 2.9 times greater than control. Interaction of the stresses showed that mechanical stress primarily controls plant water status and physiological responses. However, endophyte presence mitigated the adverse effects of individual and combined stresses on plant growth. Colonized plants were better adapted and had greater root length and volume, RWC, LWP and chlorophyll contents under stressful conditions due to higher absorption sites for water and nutrients. Compared with uncolonized plants, colonized plants showed lower CAT activity implying that wheat inoculated with *P. indica* was more tolerant and experienced less oxidative damage induced by drought and/or mechanical stress.

1. Introduction

Climate change is a major issue facing water resources in the 21st century and may increase the duration and severity of summer droughts. Therefore, drought stress is one of the most important environmental stresses which reduce plant cells' water potential and turgor, growth rate and biomass accumulation, particularly in arid and semi-arid regions. The main consequences of drought stress on plant growth and development are reduced rate of photosynthesis, cell division and elongation, root proliferation and shoot biomass and disturbed plant water and nutrient relations (Farooq et al., 2009).

Under anthropogenic climate change, many of our world's soils have become or are expected to become more vulnerable to soil degradation. Soil degradation is a major environmental problem. Physical aspects of soil degradation are mainly compaction (due to the use of heavy machinery) and increased dispersibility of soil clay (due to loss of soil organic matter and effects of mechanical disturbance). In modern mechanized agriculture, both of these

Abbreviations: PTFE, polytetrafluoroethylene; CM, complex medium; DS, drought stress; MS, mechanical stress; RFW, root fresh weight; SFW, shoot fresh weight; PH, plant height; RL, root length; RD, root diameter; RV, root volume; LA, leaf area; CART, carotenoid; Chlo *a*, chlorophyll *a*; Chlo *b*, chlorophyll *b*; Chlo T, total chlorophyll; CAT, catalase; APX, ascorbate peroxidase; RWC, relative water content; LWP, leaf water potential; ANOVA, analysis of variance.

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factors usually occur together. In physically-degraded soil, soil strength increases more rapidly with water loss due to the increase of matric suction and effective stress between soil particles (Dexter et al., 2008). A consequence of this is that as soil becomes degraded, its strength (i.e., its ability to withstand applied mechanical stresses) becomes an increasingly significant factor in limiting the growth of plant roots in soil. Therefore, drought stress and mechanical impedance can simultaneously restrict root elongation upon soil drying (Mirreh and Ketcheson, 1973). However, development of a root system is crucial for most terrestrial plants to obtain adequate water and nutrients (Bengough et al., 2006). Whalley and Clark (2011) described that only a moderate degree of soil drying can increase soil strength sufficiently to impede root elongation. It is important to realize that leaf expansion is also decreased in hard soils, due to direct signaling between root and shoot associated with the mechanical resistance.

Plants show a variety of physiological, morphological and biochemical responses to adapt themselves to stressful conditions. Plants' mechanisms to deal with water stress typically contain a mixture of stress avoidance, tolerance and recovery strategies, in which a combination of avoidance and tolerance mechanisms determines plant tolerance and adaptation capacity to drought (Malinowski and Belesky, 2000). Reduced transpirational water loss by smaller leaf area and thicker cuticle, reduced stomatal conductance, improved root water uptake with extensive and deeper root systems and maintenance of cell wall elasticity are typical responses of higher plants to water stress (Arraudeau, 1989). Oxidative stress, caused by reactive oxygen species (ROSs) as the typical secondary consequences of environmental stresses, is very dangerous for cell components and must be precisely controlled. Denaturation of DNA, amino acids and other functional and structural proteins, chlorophyll oxidation and lipid peroxidation are well-known effects of ROSs (Suzuki and Mittler, 2006). As a consequence, plant cells have evolved complex enzymatic (e.g., superoxide dismutase [SOD], CAT and APX) and non-enzymatic (e.g., ascorbic acid, glutathione and carotenoids) antioxidant mechanisms, to protect themselves from these toxic compounds (Lisar et al., 2012). The process of accumulation of low-molecular-weight osmolytes is known as osmotic adjustment. These osmolytes include proline and other amino acids, organic acids, and polyols which also play key roles in satisfying plant functions under drought (Lisar et al., 2012) and high mechanical impedance (Bengough et al., 2006). Changing the orientation of cellulose microfibrils, production of ethylene, increased sloughing of border cells and exudation from the root cap are other possible mechanisms of plant cells to deal with high soil mechanical impedance (Bengough et al., 2006). Border cells are metabolically-active cells released from the root tip as individual cells and small aggregates, or as a group of attached cells, which have the ability to engineer the chemical and physical properties of the external environment (Hawes et al., 2000; Driouich et al., 2013). Greacen and Oh (1972) suggested that the efficiencies of osmotic adjustment to compensate for increased soil mechanical resistance and lowered matric potential (<-0.8 MPa) are about 70% and 100%, respectively, in the case of pea. It means that down to a specific external matric potential, plants could fully compensate the effect of low water availability by osmoregulation. However, in the case of mechanical resistance, the compensation due to osmotic adjustment is partially effective (i.e., by 70%) (Dexter, 1987).

As the world population rises and the pressure increases on our global food system, scientists have to introduce resistant crops or identify biotic and abiotic agents to increase the resistance of plants to environmental stresses. Among biotic agents, the role of endophyte, a bacterium or fungus that lives within a plant, in stimulating host plant growth, nutrient acquisition, and improving the plant's ability to tolerate abiotic stresses is gaining more attention (Hardoim et al., 2015). *Piriformospora indica*, a root-colonizing endophytic fungus and a member of the basidiomycetous order *Sebacinales*, is originally isolated from the Indian Thar Desert. This fungus evolves mutualistic symbiosis with a wide variety of monocotyledonous and dicotyledonous plants. *P. indica* colonizes roots, forms pear-shaped spores called chlamydospores within the cortex, and does not enter vascular tissue and stems or leaves of plants (Varma et al., 2013). Recent evidence suggests that plants benefit from this relationship through increased root and shoot growth, improved osmotic adjustment and nutrient uptake, early flowering, enhanced seed production, altered antioxidative capacities and higher resistance against various biotic and abiotic stresses. Unlike mycorrhizal fungi, which cannot be cultured axenically, *P. indica* can be easily grown on synthetic or complex media without a host (Varma et al., 1999). Consequently, *P. indica* shows remarkable potential to be used as a biological agent for plant growth promotion and enhancement of plant tolerance against environmental stresses.

Several studies have shown the relationship between root system growth and individual drought or mechanical stresses. Nevertheless, there is little knowledge about the influence of combined drought and mechanical stresses on plant growth and root morphology (Dexter, 1987; Bengough et al., 2011). Moreover, the effect of *P. indica* endophytic fungus on water status, physiological characteristics and root morphology of wheat (*Triticum aestivum*), as a strategic agricultural crop, under individual and combined drought and mechanical stresses has not been studied so far. Therefore, the aim of the present study was to investigate the effects of drought and mechanical stress combinations, both with and without endophytes, on wheat growth. This enables us to better understand and potentially to mitigate problems associated with soil physical degradation and climate change.

2. Materials and methods

2.1. Preparation of plant growth medium

Sand culture medium was used to examine the effect of mechanical impedance, independent of other abiotic stresses, on plant growth. Previous studies also used sand to investigate the effect of mechanical resistance on the growth of wheat, because it allows changing mechanical impedance regardless of aeration and water content of the growing medium (Coelho Filho et al., 2013). When a weight is placed on the sand surface, the penetration resistance of growing medium is increased because the frictional resistance between sand particles is increased with negligible compaction. We used incompressible siliceous sand with a purity of 99% and a size range of 0.1–0.7 mm for the growth medium. Sand particles were washed with dilute acidic solution, then rinsed using distilled water and oven-dried at 105 °C.

2.2. Growth apparatus design and selection of weights

An adaption of the dead-load technique (Materechera et al., 1991) was used to apply different levels of mechanical impedance to the wheat seedlings. In this method, different normal stresses are applied to the incompressible sand with weights of different size.

Open-topped cylindrical Polytetrafluoroethylene (PTFE) chambers (70 mm inner diameter by 120 mm height) with removable bases were used as growth chambers (Fig. 1). The PTFE was used to reduce friction between the chamber wall and the sand so as to give a uniform distribution of mechanical stress in the chamber. Thirty six chambers (3 levels of mechanical stress × 3 levels of drought stress × 2 levels of *P. indica* inoculation × 2 replicates) were used in