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Effect of arbuscular mycorrhizal fungi inoculation on cold stress-induced oxidative damage in leaves of *Elymus nutans* Griseb

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ABSTRACT

To investigate the effect of arbuscular mycorrhizal fungi (AMF) inoculation and short-term cold exposer on *Elymus nutans* Griseb, 45 day old seedlings of two cultivars, Zhengdao (ZD) and Kangma (KM), were subjected to a 5 day cold treatment after inoculated with *Glomus mosseae*. The effects of AMF on the physiology of the two cultivars were determined, and we were especially interested in oxidative indexes under cold stress. Accumulation of reactive oxygen species (ROS), including superoxide and hydrogen peroxide, was very high in cold-stressed plants and caused lipid peroxidation in membranes, which measured as relative electrolyte leakage (REL), malondialdehyde (MDA) content. Less oxidative damage was detected in AMF colonized plants, which was associated with higher activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR). Mycorrhizal inoculum application promoted plant growth and enhanced the level of chlorophyll and antioxidant compounds such as glutathione and soluble sugars. We also measured the extent of mycorrhizal colonization. These protective mechanisms were found to be more efficient in temperature-tolerant KM than temperature-sensitive ZD. Overall, we suggest that AMF inoculation can improve plant resistance to cold stress in *E. nuatans* seedlings by directly scavenging ROS and by modulating redox balance and other defense mechanisms.

1. Introduction

Cold stress is one of the main abiotic stressors that limit growth and metabolism in plants. Accordingly, plants have evolved various cold tolerance mechanisms based on physiological and biochemical changes. Erdal (2012) showed that cold temperature can negatively affect the synthesis of photosynthetic pigments and this type of injury usually appears as etiolation in leaves. As the environmental temperature decreases, the lipids in plant cell membranes change from a liquid crystalline state to a solid-state at a critical temperature. The fatty acid phase change may give rise to cold resistance in plant cells.

Previous studies have demonstrated that oxidative stress induced by cold stress may play a crucial role in cold injury in plant cells (Li et al., 2013). Cold-related oxidative stress is thought to be mediated by reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\bullet-}$), hydroxyl radicals (·OH) and singlet oxygen ($^1O_2$) (Xu et al., 2010). To alleviate ROS injuries, complex antioxidant systems are present in plants. Antioxidants can be divided into three main classes: (1) antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR); (2) water soluble reductants such as reduced glutathione (GSH); and (3) lipid-soluble and membrane-associated molecules such as soluble sugars (Foyer and Noctor, 2011). Plants must maintain a suitable level of antioxidant activity to counteract oxidative stress. Induction of plant innate and acquired resistance is, therefore, vital and necessary under stress conditions.

The term mycorrhiza is used to describe the mutualistic symbiotic relationship that takes place between the plant root and colonizing fungi. Ectomycorrhizal fungi are found mainly among the roots of trees in temperate forests, and live outside plant cells. Endomycorrhiza, including arbuscular mycorrhiza (AM), insert part of the fungal hyphae inside the plant cell. AM is probably the most widespread form of symbiosis (Parniske, 2008) and has been estimated to be present in more than 80% of terrestrial plant species. Arbuscular mycorrhizal fungi (AMF) are obligate symbionts and acquire carbon from their host plants to complete their life cycles (Bago et al., 2000). In return, the fungi provide multiple benefits for the plants, including enhanced mineral nutrition and tolerance to abiotic and biotic stresses (Sawers et al., 2008). Not only do they benefit plant with their growth and development (Abbott and Robson, 1982; Yooyongwech et al., 2013) they also increase resistance to stresses, such as, drought (Bárzana

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et al., 2014), salinity (Porcel et al. 2012), heavy metals (Gamalero et al., 2009), pathogens (Veresoglou and Rillig, 2012), and cold temperature (Chen et al., 2013).

The Qinghai-Tibet Plateau spans approximately 2.5 million km^2 and has an average altitude of 4,000 m. About 35% of its area consists of alpine meadow, where plant growth is limited by the extreme climate. *Elymus nutans* is widely distributed on the Qinghai-Tibet Plateau. Its moderate nutrient content and palatability make it a food source for most alpine animals, especially the yaks.

In our study, we evaluated the effect of Glomus mosseae on *E. nutans* Griseb plant growth, photosynthetic pigment content, membrane lipid peroxidation, and antioxidant enzyme activity in the shoot of the Kangma (KM) and Zhengdao (ZD) cultivars of *E. nutans* under cold stress. The KM cultivar was found to stronger cold resistance after AMF inoculation than the ZD cultivar, and it is worth mentioning that the ZD cultivar is a product of artificial breeding while the KM cultivar was obtained from the Qinghai-Tibet Plateau. We also succeeded in further elucidating mechanisms for cold resistance in mycorrhizal plants. Specifically, we hypothesized that: (1) Improvements in plant growth and development are relied on AMF inoculation; (2) AMF would enhance the capacity of two *E. nutans* cultivars to resist cold stress.

2. Materials and methods

2.1. Mycorrhizal inoculum

Mycorrhizal inoculum was provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forest Sciences, China. The inoculum consisted of soil, spores (the spores density was approximately 30,000 per 20 g inoculums), hyphae, and infected root fragments from a stock culture of *G. mosseae*.

2.2. Plant material and treatments

E. nutans seeds were obtained from two sources: seeds of Kangma (KM) were collected in September 2012, from wild plants growing in Kangma County (28°33′N, 89°40′E, altitude 4,359 m), located in the middle of Tibet, China. Agriculture and Animal Husbandry Bureau in Tibet responses for Kangma County. *E. nutans* occurs naturally and abundantly at altitudes between 3,000 and 5,000 m in the Qinghai-Tibetan Plateau, the field studies did not involve endangered or protected species. *E. nutans* (Zhengdao, ZD) seeds were obtained from Beijing Rytway Ecotechnology Co., Ltd. (116° 33′N, 40° 35′E, altitude 550 m), Changping District, Beijing, China in September 2012. Seeds were cleaned and stored at 4 °C in paper bags until the start of the experiments.

Seed of *E. nutans* (ZD and KM) were surface sterilized in 0.1% (*w*/*v*) sodium hypochlorite, rinsed several times in distilled water, and germinated on moistened filter paper at room temperature for 7 days. Seedlings were selected and transferred into 100 mL black plastic pots containing 80 mL of quartz sand. One third of these seedlings were inoculated with 2 g activated mycorrhizal inoculum, and the other 1/3 were inoculated with equal quality of sterilized mycorrhizal inoculum, which were placed 2 cm below the *E. nutans* seeds at sowing time, the rest of them were cultivated as control group without inoculation.

Seedling cultured by nutrient solution containing 4 mM $Ca(NO_3)_2$, 4 mM KNO_3, 1 mM KH_2PO_4, 2 mM $MgSO_4$, 46 μM H_3BO_3, 10 μM $MnSO_4$, 50 μM Fe-EDTA, 1.0 μM $ZnSO_4$, 0.05 μM H_2MoO_4 and 0.95 μM $CuSO_4$. Nutrient solution pH was adjusted close to 6.5 by adding H_2SO_4 or KOH, and watered every three days. The plants were grown in a growth chamber at a day/night temperature of 25/20 °C, a relative day/night humidity of 70/65%, a day/night of 16/8 h and a photosynthetic photon flux density (PPFD) at the height of the plant of 100 μmol m^{-2} s^{-1}. Light was provided by a fluorescent lamp.

Halves of 45-day-old plants, which from three different kinds of culture substrates, were exposed to cold condition at a day/night temperature of 5/5 °C, a relative day/night humidity of 60/65%, a day/night of 16/8 h and a PPFD of 100 μmol m^{-2} s^{-1}. After 5 days, plants for each treatment were sampled and frozen in liquid nitrogen, and then stored at −80 °C for further analysis.

2.3. Plant growth performance measurement

To detect the extent of mycorrhizal colonization, a fraction of the roots was washed carefully, and then cut into 1 cm long segments, dipped in 10% KOH at 90 °C for 20 min, acidified in 2% HCl for 5 min, and stained with 0.05% acid fuchsin in lactic acid, with a slight modified

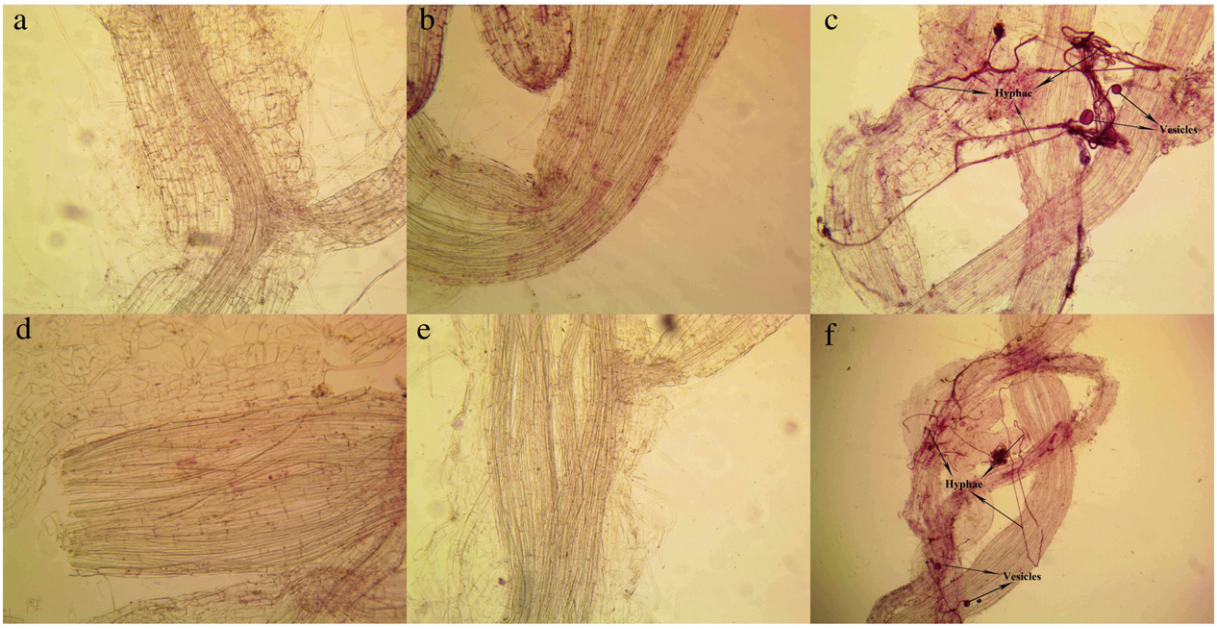

Fig. 1. The extent of mycorrhizal colonization in roots of *E. nutans* under cold stress. The Zhengdao cultivar (ZD) without inoculation (a), ZD inoculated with sterilized mycorrhizal inoculum (b), ZD inoculated with activated mycorrhizal inoculum (c);The Kangma cultivar (KM) without inoculation (d), KM inoculated with sterilized mycorrhizal inoculum (e), KM inoculated with activated mycorrhizal inoculum (f).