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SHORT NOTE

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Influence of water stress on biomass production by isolates of an ericoid mycorrhizal endophyte of *Woollsia pungens* and *Epacris microphylla* (Ericaceae)

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Abstract The influence of water stress on growth of six isolates of an anamorphic ericoid mycorrhizal fungal endophyte from two Australian Ericaceae hosts was investigated. Biomass production was determined in liquid media amended with NaCl or polyethylene glycol to give osmotic potentials of –0.05 to –2.24 MPa. Regardless of the osmoticum, biomass yields of all isolates at the highest level of imposed water stress (–2.24 MPa) did not differ significantly from, or were significantly higher than, those at the lowest level of water stress. The data suggest that the endophyte isolates possess considerable resistance to water stress and may be of functional importance to their plant hosts under water-stress conditions.

Keywords Ericoid mycorrhizal fungi · Epacrids · Water stress · Salinity

Introduction

Plants that form ericoid mycorrhizas (ERM), including epacrids and other Ericaceae, are common components of heathland and some forest habitats in both northern and southern hemispheres (Specht 1979; Read 1991). Typically they occur on soils of extremely low nutrient status that display various combinations of low pH, high metal availability, poor drainage, free drainage and high or low temperatures (Read 1996). ERM associations are thought to be important in the establishment and survival of Ericaceae plants in these stressful environments (Cairney and Meharg 2003).

Fungi that form ERM with epacrids in the southern hemisphere have been isolated only as sterile mycelia. Molecular analyses suggest that, in common with those from northern hemisphere Ericaceae, most have taxonomic affinity with Helotiales ascomycetes (see Cairney and Ashford 2002). The ability of ERM fungi to facilitate survival of northern hemisphere Ericaceae under conditions of nutrient stress has received considerable attention. This appears largely to be related to their abilities to produce a broad range of hydrolytic and oxidative enzymes that are important in mobilising nutrients from organic complexes (reviewed in Cairney and Burke 1998). In some Ericaceae habitats, such as low-pH heathlands, serpentine soils and metalliferous mine spoils, soils contain high levels of potentially toxic metals and metalloids. There is convincing evidence that ERM fungi from heathlands and metal-contaminated sites in the northern hemisphere have considerable resistance to metals and metalloids (Bradley et al. 1981; Burt et al. 1996; Martino et al. 2000; Sharples et al. 2000). While studied in less detail, ERM endophytes from Australian epacrids appear to be broadly similar to those from northern hemisphere Ericaceae in terms of both their abilities to utilise nutrients in organic forms and their resistance to toxic metals (Cairney and Ashford 2002). The ability of ERM endophytes to withstand other edaphic stresses remains to be tested.

Many of the Australian habitats occupied by epacrids are subject to periodic water stress, either annually or, in south-eastern Australia, during longer periods of drought resulting from the El Niño Southern Oscillation. They may also be affected by water stress and ionic stresses associated with soil salinity. Despite the importance of water stress in these environments, little is known currently regarding the responses of ERM endophytes to water stress. In the only study so far conducted, Hutton et al. (1996) reported varied responses of putative ERM endophytes to induced water stress. Some isolates showed decreased radial growth with increasing water stress, while others grew better under increased water stress than under minimal water stress (Hutton et al. 1996). No clear

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relationship was found, however, between isolate response to water stress and the degree of water stress predicted for their habitats of origin. In order to investigate resistance to water stress in more detail, we studied the responses of six genotypes of an ERM endophyte isolated from two epacrid taxa at a dry sclerophyll forest site and a coastal heathland site in eastern Australia.

Materials and methods

The fungi used in the study were anamorphic isolates of a taxon from epacrid roots that, based on rDNA internal transcribed sequence data, has affinity with the Helotiales ascomycetes of the *Hymenoscyphus* complex (Midgley et al. 2002). The taxon has been shown to form typical ERM coils in hair roots of *Woollsia pungens* (Cav.) F.Muell. under gnotobiotic conditions (D.J. Midgley, S.M. Chambers, J.W.G. Cairney, unpublished data) and is thus regarded as an ERM endophyte. Four isolates (MG1, MG8, MG41, MG46) were obtained from hair roots of a single *W. pungens* plant at a dry sclerophyll forest site in the Blue Mountains National Park, NSW, Australia (33° 42' S, 151° 08' E) and two (EF7, EF8) from separate *Epacris microphylla* R. Br. plants at a coastal heathland site in Brisbane Waters National Park, NSW (33° 32' S, 151° 16' E). Inter-simple sequence repeat PCR has confirmed that each isolate is a different genotype (Midgley et al. 2002). All endophytes were initially isolated during 1999 and were routinely maintained on 2% malt extract agar in the dark with subculturing every 4–6 weeks.

The influence of water stress on biomass production by the endophytes was examined in liquid culture by comparing biomass production in a basal growth medium and the same medium amended with NaCl or polyethylene glycol (PEG) at different concentrations. The basal medium was a modified version of Modified Melin Norkans (MMN) medium (Marx and Bryan 1975) containing (l^{-1}): $(NH_4)_2HPO_4$, 500 mg; KH_2PO_4, 300 mg; glucose, 10 g; $MgSO_4.7H_2O$, 140 mg; $CaCl_2$, 50 mg; NaCl, 25 mg; $ZnSO_4$, 3 mg; thiamine, 0.133 mg; ferric EDTA, 12.5 mg. NaCl or PEG 6000 (Sigma) was added to give osmotic potentials of –0.05 (10 mM NaCl, 1.0 mM PEG), –0.23 (50 mM NaCl, 6.0 mM PEG), –0.45 (100 mM NaCl, 11 mM PEG), –0.67 (150 mM NaCl, 16 mM PEG), –0.90 (200 mM NaCl, 20 mM PEG), –1.12 (250 mM NaCl, 27 mM PEG), –1.34 (300 mM NaCl, 31 mM PEG), –1.79 (400 mM NaCl, 40 mM PEG) and –2.24 MPa (500 mM NaCl, 51 mM PEG) (calculated according to Slavík 1974). Media were adjusted to pH 5–5.5 prior to the addition of the ferric EDTA and autoclaving. Discs of inoculum (5 mm diameter) were cut from the actively growing colonies on MMN agar medium (Marx and Bryan 1975) (inoculated 3 weeks previously) and one disc for each isolate was inoculated into 9-cm-diameter Petri dishes containing 25 ml liquid medium. Cultures were incubated at 23°C in the dark for 14 days and each treatment was replicated five times. Following incubation, mycelial mats were removed from the liquid media, dried overnight at 80°C and biomass determined gravimetrically. Data for each isolate and osmoticum were analysed by one-way ANOVA and significant differences determined by Fishers PLSD.

Results and discussion

All isolates produced measurable biomass at all levels of water stress resulting from inclusion of either NaCl or PEG in the medium (Fig. 1). With either osmoticum, biomass yields at the highest level of imposed water stress (–2.24 MPa) were not significantly lower ($P > 0.05$) than yields at the lowest water stress (unamended MMN) for all isolates (Fig. 1). Indeed, in the cases of isolates MG41 (NaCl treatment) and EF7 (NaCl and PEG treatments), biomass production was significantly higher ($P > 0.05$) at –2.24 MPa than in unamended MMN (Fig. 1). In the NaCl treatments, all isolates showed a peak in biomass production at –1.34 MPa, which (except for MG41) was significantly higher ($P > 0.05$) than at any of the other water stress levels (Fig. 1a). In contrast, while slight peaks in biomass production were variously observed for some isolates at certain water stress levels with PEG as osmoticum, no consistent patterns were observed for any of the isolates (Fig. 1b). Peaks in biomass production at intermediate NaCl concentrations have been observed previously for ECM fungi (Saleh-Rastin 1976; Dixon et al. 1993; Chen et al. 2001). While it has been suggested that such peaks reflect responses to external water potential (Chen et al. 2001), the absence of the peaks in the equivalent PEG treatment implies that an ionic effect of the NaCl treatment is a more likely explanation.

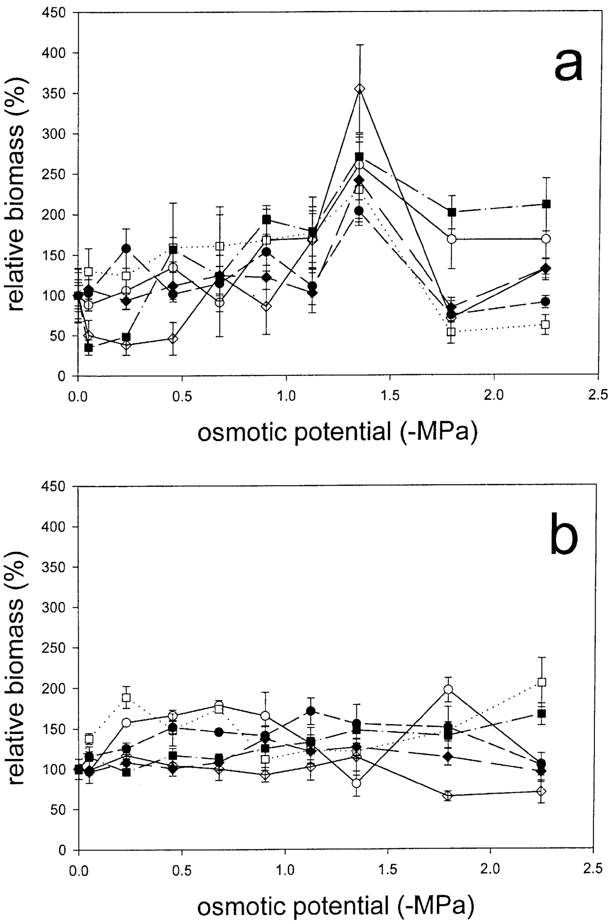

Fig. 1 Influence of decreasing water potential, induced by **a** NaCl or **b** PEG 6000, on biomass production by six isolates of the ericoid mycorrhizal fungal endophyte of *Woollsia pungens* over a 14-day growth period (*MG1* ○, *MG8* ◇, *MG41* □, *MG46* ●, *EF7* ■, *EF8* ◆)

The data indicate that growth of the ERM endophyte taxon is little affected by NaCl up to a concentration of ca. 500 mM, which is well in excess of that typically found in saline soils (Peck 1993). All isolates showed broadly similar growth patterns in the presence of NaCl, suggesting that little intraspecific variation exists in this respect. The isolates from the sclerophyll forest site thus appear to be as resistant to NaCl as those from the coastal heathland site, at least over the concentration range investigated. In the only other investigation of the effect of NaCl on ERM fungi conducted to date, Rice and Currah (2001) reported that ERM strains of the Onygenales ascomycete *Oidiodendron maius* Barron from a range of habitats continued growth at ca. –2.92 MPa, albeit reduced considerably compared with unamended medium.

Although responses to NaCl of a broader range of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi have been studied, these have been limited to a relatively narrow concentration range (equivalent to approximately 0.90 MPa) (Saleh-Rastin 1976; Dixon et al. 1993; McMillen et al. 1998; Chen et al. 2001). Chen et al. (2001), however, found that growth of some *Pisolithus* spp. isolates from Australian sclerophyll forest habitats was not significantly reduced at 200 mM NaCl (ca. 0.90 MPa) compared with unamended MMN, suggesting that growth would have continued at higher concentrations had they been included in their experiment.

Polyethylene glycol has been used widely as an osmoticum in investigations of water stress, since it is not metabolised by fungi, has low toxicity and provides both osmotic and matrix components of water stress (Mexal and Reid 1973; Coleman et al. 1989). Hutton et al. (1996) reported variation in putative ERM fungi from Western Australian epacrids with respect to their resistance to PEG-induced water stress. Growth of these isolates, however, appeared to be reduced at –2.96 MPa compared with unamended MMN, suggesting lower resistance to water stress than the isolates screened in the present study. Unfortunately, the taxonomic affinities of these isolates could not be ascertained.

Investigations of ECM fungi have reported both intra- and interspecific variation in responses to PEG-induced water stress. Mexal and Reid (1973) found that while some *Cenococcum graniforme* (= *C. geophilium*) isolates grew best at –1.5 MPa, these grew poorly at –2.0 MPa. In screening a broad taxonomic range of ECM fungi, Coleman et al. (1989) reported that many were able to grow at –2.96 MPa, but that growth was reduced significantly compared with that in unamended MMN. For AM fungi, significantly reduced growth is common at PEG-induced water stress well below –3.0 MPa (see Juniper and Abbott 1993). The anamorphic ERM endophyte isolates from *W. pungens* thus appear to be more resistant to water stress than other mycorrhizal fungi screened so far. Significantly, some unidentified anamorphic fungi isolated from ECM roots in coniferous forests in Oregon, USA, but thought not to be ECM fungi, were found to grow as well or slightly better at –2.96 MPa than in unamended MMN (Coleman et al. 1989). This parallels our observations for the *W. pungens* endophyte isolates and is consistent with previous observations that anamorphic fungi often possess significant resistance to water stress (e.g. Tresner and Hayes 1971; Griffin 1972). While the ability of *W. pungens* to withstand water stress and the ability of its ERM endophytes to confer benefits upon the host under water stress conditions remain to be investigated, our data indicate that the endophytes have the potential to function under conditions of extreme water stress.

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