

ROLE OF TREHALOSE IN DESICCATION TOLERANCE OF ENDOPHYTE-INFECTED TALL FESCUE

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IMPACT STATEMENT

A fungal endophyte (*Neotyphodium coenophialum*) that naturally infects tall fescue (*Festuca arundinacea*) enhances host survival under heat and drought conditions compared with non-infected plants. However, the mechanism for this enhanced drought tolerance is unclear. Trehalose, a disaccharide, is known to accumulate in desiccation-tolerant plants, fungi, and invertebrates, where it protects these organisms under severe stress conditions. To assess the role of trehalose in desiccation protection of endophyte-infected tall fescue, carbohydrate analysis of endophyte cultures and endophyte-infected tall fescue plants was conducted. Endophyte cultures that were subjected to water deficit stress exhibited changes in growth and carbohydrate profiles. Field studies comparing well-watered and water-stressed plants demonstrated similar changes in carbohydrate profiles.

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BACKGROUND

Endophytes are symbiotic fungi found in many cool-season grasses and have been shown to increase drought tolerance, insect and disease resistance, and general host vigor, especially under stress conditions. In tall fescue infected with *N. coenophialum*, enhanced drought tolerance is well documented. However, the basic physiological mechanisms are unclear.

Osmotic adjustment, or osmoregulation, is the accumulation of compatible, soluble solutes in response to long-term water deficit. Osmotically active compounds include non-structural, soluble carbohydrates, ions such as potassium, calcium, and sodium, and proteins and amino acids such as glycine-betain and proline. The major soluble carbohydrates in tall fescue are polymeric fructans, the disaccharide sucrose, and the monosaccharides fructose and glucose. In the early 1990s, Richardson and Bacon (1994) identified sugar alcohols associated with the endophytic fungus infecting tall fescue. These sugar alcohols or polyols—mannitol and arabitol—are common osmolytes in fungi.

Another carbohydrate commonly found in fungi and a broad range of desiccation-tolerant organisms is trehalose, a disaccharide (consisting of two glucose molecules). Trehalose is found in fungi such as dry active baker's yeast *Saccharomyces cerevisiae*, slime mold macrocysts *Dictyostelium*, and certain fungal spores. Several plant species known as resurrection plants (*Sporobolus stapfianus*, *Selaginella lepidophylla*, and *Myrothamnus flabellifolius*) contain high levels of trehalose and can survive extended periods of desiccation. On the basis of the widespread occurrence of trehalose in fungal systems and the documented effects of fungal endophytes on water deficit stress in tall fescue, studies were conducted to determine whether trehalose is present in endophyte-infected tall fescue and in endophytes grown in vitro.

RESEARCH DESCRIPTION

Carbohydrate analysis. Carbohydrates were analyzed following the protocol of Chapman and Horvat (1989). After samples were freeze-dried and ground to a powder, soluble carbohydrates were extracted with 75% ethanol. Soluble carbohydrates were converted to their volatile, oxime-trimethylsilyl (TMS) derivatives and separated on a gas chromatograph. Elution peaks were identified by comparison to known standards and were compared with a synthetic sugar standard, phenyl β -D-glucoside, for quantification.

Field study. To investigate the presence of trehalose in endophyte-

infected tall fescue, a rainout shelter at the University of Arkansas Agricultural Research and Extension Center, Fayetteville, was utilized to induce a uniform drought event. Populations from the cultivar 'HiMag' were used in this trial. One population was an endophyte-free control (HM-), and three populations (HM4, HM8, and HM9) were inoculated with unique endophyte (*N. coenophialum*) strains isolated from drought-resistant hosts. The statistical design was a split-plot design with two irrigation regimes, well-watered and drought, and six replications of the four 'HiMag' populations in each water regime. The dry-down period was initiated on 3 June 1999. Fifty-eight days later, when drought plots showed severe signs of water stress, leaf pseudostems were harvested 1 cm above the meristem and immediately frozen in liquid nitrogen for subsequent carbohydrate analysis. At the time of harvest, average water potential of the drought-stressed plots was -4.6 MPa and turgor potential was 0.4 Mpa, while well-watered plots had a water potential of -0.5 MPa and turgor potential of 1.4 MPa. Differences in carbohydrate levels were analyzed with use of analysis of variance procedures.

Endophyte culture. To determine whether trehalose is a component of endophyte (*N. coenophialum*) metabolism, one of the endophyte strains inoculated into the 'HiMag' population was cultured on osmotically adjusted potato dextrose agar (PDA). The endophyte inoculated into HM8, designated E8, was re-isolated from its host and maintained on PDA at 21°C in darkness. When cultures reached a diameter of 1.5 to 2 cm, discs were cut from the endophyte culture and homogenized in sterile water. An aliquot (1.75 mL) of the homogenized mycelium was used to inoculate the water-stress medium, which consisted of PDA amended with glycerol to simulate water potentials of 0 , -0.3 , -0.7 , and -1.3 Mpa. Prior to inoculation, a sterile cellophane membrane was laid over the solidified medium. After 34 days, cultures were harvested from the cellophane membrane and analyzed for soluble carbohydrate content. Changes in carbohydrate content were statistically analyzed using the SAS GLM procedure.

FINDINGS

When grown on osmotically adjusted PDA, endophyte E8 produced consistent levels of trehalose, but levels were low compared with arabinol and mannitol (Fig. 1). At moderate water stress, -0.7 MPa, mannitol and arabinol were at significantly higher levels than trehalose and may be acting as osmoregulators. Richardson et al. (1992) also reported the presence of arabinol and mannitol in endophyte cultures but did not report trehalose in those studies. Although the amount

produced is low, this is the first report of the presence of trehalose in the endophyte of tall fescue. In general, this endophyte had poor growth on all the media, but growth of the endophyte was significantly greater on the -0.3, -0.7, and -1.3 MPa media than on the 0 MPa medium, with -1.3 MPa medium having the greatest dry weight increase (data not shown). This indicates that optimal growth occurs not in water-saturated conditions but under slight-to-moderate water tension.

In the field studies, there were significant differences in plant carbohydrates between well-watered and drought-stressed plots (Table 1). However, there were no significant endophyte effects on any plant-derived carbohydrates. Sucrose increased in all drought-stressed plots, which would support its role as an osmoregulant in many higher plants.

There were no significant differences between fungal carbohydrates in well-watered versus drought-stressed plots. Interestingly, arabinol was not present in any of the endophyte-infected 'HiMag' populations (Table 1), although it was present in the endophyte strain E8 tested in vitro on PDA (Figure 1). In addition, arabinol was previously reported by Richardson et al. (1992) in an endophyte-infected tall fescue ('Kentucky 31') plant. Mannitol, a fungal-produced carbohydrate, was present in endophyte-infected plants and not in endophyte-free plants. Similarly, trehalose was present only in endophyte-infected, drought-stressed plants. Under well-watered conditions, some HM- plots also had trehalose present. Although this requires further investigation, the presence of trehalose on endophyte-free plots may be explained by fungal contamination from *Rhizotonia solani*, which was prevalent on all of the well-watered plots but not on the drought-stressed plots.

It has been predicted that the total biomass of the endophyte is less than 1% of the total plant biomass, which would explain why the fungal carbohydrate pool is much lower than the plant carbohydrate pool (Table 1). This dilution effect would suggest that the fungal contribution to the total osmotic pool has a lesser role. Concentrations of trehalose in endophyte-infected plants appear to be too low to act as an osmoregulator. However, trehalose also has a non-osmotic role in stressed organisms, where it effectively stabilizes dry membranes, phospholipid bilayers, and proteins (Crowe et al., 1987; Crowe et al., 1993). Bilayer membranes are composed of fatty acid derivatives with long hydrophobic tails and hydrophilic head groups, which helps them to form sheet-like structures surrounding cells and organelles. In a normal aqueous environment, the hydrophobic tails on the bilayer interior are in a semi-fluid state. When membranes are desiccated or frozen, the tails become rigid and break when rehydrated or thawed. Trehalose binds to the hydrophilic head groups on the exterior of the membrane and stabilizes the semi-fluid state of the membrane during

desiccation. Crowe et al. (1987) found that proteins and membranes that were dehydrated in the presence of trehalose were fully functional when rehydrated.

In conclusion, trehalose was identified in both endophyte-infected tall fescue plants and cultures of *Neotyphodium* endophytes. Although the quantities present in these studies suggest an insignificant effect on osmotic potential, trehalose may serve to protect membranes under severe desiccation.

LITERATURE CITED

- Chapman, G.W., and R.J. Horvat. 1989. Determination of nonvolatile acids and sugars from fruits and sweet potato extracts by capillary GLC and GLC/MS. *J. Agric. Food Chem.* 37:947-950.
- Crowe, J.H., L.M. Crowe, S.B. Leslie, and E. Fisk. 1993. Mechanisms of dry biomolecules in anhydrobiotic organisms. In: Close, T.J. and E.A. Bray, eds. *Plant responses to cellular dehydration during environmental stress*. Rockville, Md.: American Society of Plant Physiologists. pp. 11-20.
- Crowe, J.H., L.M. Crowe, J.F. Carpenter, and C. Aurell Wistrom. 1987. Stabilization of dry phospholipid bilayers and proteins by sugars. *Biochem. J.* 242:1-10.
- Richardson, M.D., and C.W. Bacon. 1994. Stress tolerance of endophyte-infected turfgrasses. In: Bacon, C.W. and J.F. White, Jr., eds. *Biotechnology of endophytic fungi of grasses*. Boca Raton, Fla.: CRC Press. (p. 515-523)
- Richardson, M.D., G.W. Chapman, Jr., C.S. Hoveland, and C.W. Bacon. 1992. Sugar alcohols in endophyte-infected tall fescue under drought. *Crop Sci.* 32:1060-1061.

Table 1. Gas chromatography results of soluble carbohydrates in endophyte-infected tall fescue pseudostems harvested 31 July 1999 from the rainout shelter at the University of Arkansas Agricultural Research and Extension Center, Fayetteville.

Endophyte	Plant carbohydrates			Fungal carbohydrates		
	Sucrose	Glucose	Fructose	Arabitol	Mannitol	Trehalose
	mg/g dry weight					
	Drought-stressed					
HM-	142.9	28.9	19.9	0	0	0
HM4	173.4	28.9	19.0	0	0.6	1.4
HM8	179.9	27.0	15.3	0	0.1	0.7
HM9	166.7	27.8	16.8	0	0.1	0.4
	Well-watered					
HM-	44.2	32.3	26.3	0	0	0.8
HM4	48.5	30.0	23.5	0	1.2	0.6
HM8	47.0	33.0	25.6	0	0.3	0.2
HM9	48.8	35.4	27.6	0	0.1	0.2
Water regime ^z	**	NS	NS	***	**	***
Endophyte	**	***	NS	NS	NS	NS
Endo x Water	**	NS	NS	NS	NS	NS

^z NS, not significant at $P = 0.05$; ** $P > 0.01$; *** $P > 0.001$.

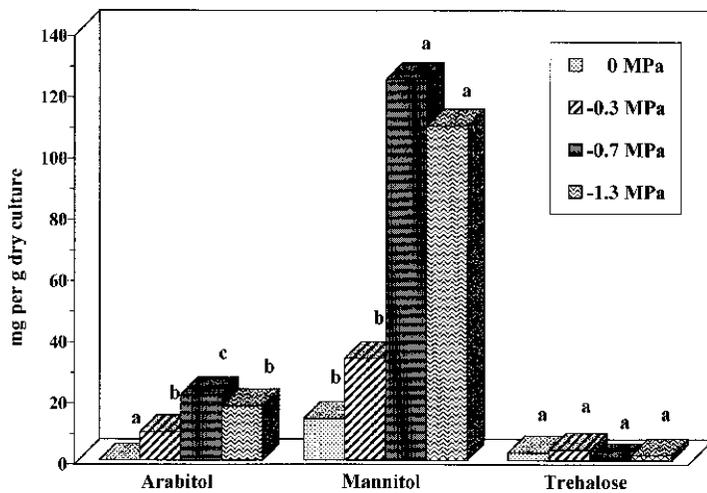


Fig. 1. Soluble carbohydrates extracted from endophyte 8 grown on osmotically adjusted potato dextrose agar.