Biochemical Responses to Water Deficit in Summer Dormant and Summer Active Tall Fescue

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ABSTRACT

Water deficit from summer drought can severely limit growth of cool-season perennial grasses; however, some species have developed summer dormancy as a trait to survive drought stress. The role of biochemical regulation under drought conditions in summer-dormant versus summer-dormant tall fescue is not clearly understood. Two incompletely summer-dormant tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh.] populations, and one summer-active cultivar, each with and without endophyte, were grown under non-irrigated conditions in field plots at a humid site and evaluated for dehydrin expression, superoxide dismutase activity and total phenolic concentration and plant survival. The experiment was replicated at a semi-arid site where plant survival and phenolic concentrations were evaluated. The summer-dormant populations achieved 96-99% survival, but had reduced responsiveness of biochemical traits to drought. The summer-active cultivar had greater biochemical responsiveness than the summer-dormant populations but lower survival (83% at the humid site and 36% at the semi-arid site). Summer dormancy and high survival was linked to reduced biochemical activity. This result is consistent with the lack of tiller growth, decreased water content, and increased leaf senescence observed in these plants.

INTRODUCTION

Drought resistant cultivars of tall fescue, whose germplasm originates in the Mediterranean region, have developed varying degrees of summer dormancy, a trait which forces a slowdown of growth even in the event of summer rains. This trait allows the plant to avoid drought and extend survival until autumn recovery. In contrast, summer-active tall fescue cultivars currently recommended for forage production have limited persistence due to drought, especially when lacking its endophyte (*Neotyphodium coenophialum*). Dehydrin proteins, superoxide dismutase (SOD), and phenolics can contribute to cell protection from oxidative stress and are hypothesized to improve drought survival. Volaire et al. (1998) reported that dehydrin accumulation was associated with enhanced survival of grass meristematic tissue during drought stress. Jiang and Huang (2001) found higher dehydrin accumulation associated with reduced dehydration in two tall fescue turf cultivars. Fu and Huang (2001) detected increased SOD activity in tall fescue and Kentucky bluegrass (*Poa pratensis* L.) during initial drying periods and reported that potent antioxidants such as phenolics protect plant cells from oxidative damage caused by abiotic stress. The objective of this study was to examine these biochemical traits and determine their association with summer dormancy and summer activity in tall fescue during drought.

MATERIALS AND METHODS

Field experiments were conducted at a humid site at the Agricultural Research and Extension Center, Fayetteville, Arkansas (36°N, 94°W), and at a semi-arid site at the Texas AgriLIFE Center, Vernon, Texas (34°N, 99°W). Incompletely summer-dormant populations, TX06V-B-FA (TX) and 'Grasslands Flecha' and one summer-active cultivar, 'Kentucky 31' (KY), with and without endophyte, were established in field trials. Only data from non-irrigated plots will be reported. Tall fescue tillers were harvested at early, mid- and late summer sampling times during the summers of 2007 and 2008 and assayed for dehydrins, SOD, and total phenolics for

Fayetteville, and for phenolics for Vernon. Live tillers were counted in late spring and mid-autumn to determine the percentage tiller survival rate. Dehydrins and SOD were analyzed in the basal 2 cm of tillers, and phenolics were analyzed in leaf tissue above 2 cm.

Total protein was extracted and dehydrins were detected using western hybridization (Close et al. 1993). Electrophoresis and western hybridization was performed with an antidehydrin polyclonal antibody and then with the secondary antibody, a goat anti-rabbit fragment and detected using the Bio-Rad Immunstar^{TM} chemiluminescence system. As outlined by McCord and Fridovich (1969), superoxide dismutase was assayed by adding xanthine oxidase to xanthine, cytochrome *c* and stressed total fescue protein. Absorbance of SOD activity was recorded at 550 nm from a spectrophotometer. Phenolics were extracted from freeze-dried leaf tissue with methanol and the Price and Butler method was performed (Waterman and Mole, 1994). A range of standards (0 to 2 µg/mL) was prepared with phenol and the total phenolic concentration was calculated from a standard calibration curve (Malinowski et al., 1998). Data were converted to tannic acid equivalents.

RESULTS AND DISCUSSION

Drought-stressed KY accumulated the highest intensity of dehydrin transcripts among the three populations, with Flecha at intermediate levels, and TX showing the least accumulation during both summers (data not shown). Norton et al. (2006) found that as summer drought progressed, 'Demeter', a summer-active tall fescue cultivar, accumulated higher levels of dehydrin transcripts than did Flecha. Dehydrin accumulation did not decline in Flecha after a summer storm even though tiller-base water content increased and dehydrin levels of Demeter declined. Both studies indicate that dehydrin production is less responsive to water supply in summer dormant populations, whereas it is up-regulated in summer-active plants during drought stress.

There was no apparent endophyte effect on dehydrin accumulation in our trials. Guerber et al. (2007) also detected no endophyte differences in dehydrin patterns in a tall fescue genotype originally from a xeric environment, but did detect an endophyte enhancement of dehydrin expression in a mesic-origin genotype. These results suggest that genotypes can xeric-origin genotypes (presumably possessing some degree of summer dormancy) can survive drought conditions independent of endophyte- or dehydrin-mediated protection.

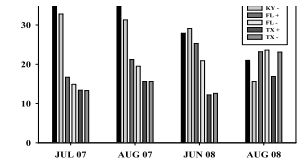

Figure 1. Superoxide dismutase (SOD) activity in tillers of non-irrigated tall fescue for 2007 and 2008. Means were averaged across three replications. Endophyte status indicated by + (with) and – (without). KY=KY31, FL=Flecha, TX=TX06V-B-FA.

During drought periods in 2007 and June 2008, KY had higher of SOD activity than summer-dormant populations (Fig. 1). Differences were not detected in August 2008, an abnormally wet month. Lower SOD activity in the summer-dormant populations than in KY indicated Flecha and TX were producing less reactive oxygen species, which likely was a consequence of being less metabolically responsive to drought. Volaire et al. (2005) found that monosaccharide

concentrations in drought-stressed orchardgrass (*Dactylis glomerata* L.) were negatively associated with the degree of summer-dormancy expression, again suggesting that metabolic activity is reduced in the more-dormant grasses.

At the humid site (Fayetteville) during both summers and at the semiarid site (Vernon) during the summer of 2007 and June 2008, total free phenolic concentrations followed the same trends as dehydrins and SOD (Fig. 2) in that the summer-dormant populations produced lower phenolic concentrations than did KY. The exceptions to this trend were in the July and August 2008 samplings at Vernon, where Flecha produced significantly higher concentrations of phenolics. By autumn 2008 across sites, tiller survival rate averaged 60% in KY, 96% in Flecha, and 99% in TX (see Underwood et al., this proceedings for details on survival data). Therefore, superior survival of summer dormant plants was not associated with enhanced biochemical protectants; indeed the opposite occurred. Biochemical protectants are more metabolically responsive to summer drought in summer-active tall fescue than in summer dormant types.

No significant endophyte differences were found at the humid site. At the semiarid site, however, endophyte-positive KY produced significantly (P<0.05) higher phenolic concentrations than endophyte-free KY on most sampling dates. This agrees with the finding by Malinowski et al. (1998) that endophyte-positive tall fescue plants produced higher phenolic concentrations than endophyte-free plants. Malinowski and Belesky (2000) proposed that symbiotic interactions between the endophyte and its host may activate defense mechanisms in the host, which in turn is manifested by increased levels of phenolic compounds.

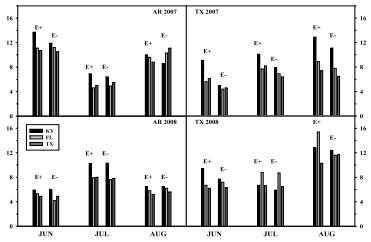

Figure 2. Phenolic concentrations in tillers of three drought-stressed tall fescue populations in a humid site (AR) and a semi-arid site (TX) in 2007 and 2008. Means were averaged over four replications. KY= KY31, FL=Flecha, TX= TX06V-B-FA.

CONCLUSIONS

There was generally an inverse relationship between the propensity of a tall fescue population for summer dormancy (TX being most summer dormant and KY being least; see West et al., this proceedings for dormancy ranking of these populations) and expression of dehydrins, SOD, and total phenolics. Moreover, the population that exhibited the greatest expression of biochemical protectants and least dormancy (KY), also had the lowest tiller survival after drought. We propose that reduced expressions of putative biochemical protectants in summer-dormant relative to summer-active tall fescue i) are another manifestation of reduced metabolic activity associated

with the summer-dormancy trait, and ii) suggest that antioxidants may serve a more beneficial function in summer-active than in summer-dormant tall fescue.

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