Differential accumulation of dehydrins in response to water stress for hybrid and common bermudagrass genotypes differing in drought tolerance

Longxing Hu [a], Zhaolong Wang [a,*], Hongmei Du [a], Bingru Huang [b,*]

[a] College of Agricultural and Biological Science, Shanghai Jiao Tong University, China
[b] Department of Plant Biology and Pathology, Rutgers University, New Brunswick, NJ 08901, USA

ABSTRACT

Expression of dehydrin proteins may be induced or enhanced by environmental stresses that lead to cell dehydration. The objective of the this study was to investigate genetic variation in dehydrin protein accumulation in response to drought stress of whole-plants or dehydration of detached leaves and to identify dehydrins differentially expressed in bermudagrass (Cynodon spp.) genotypes differing in drought tolerance. Plants of four hybrid bermudagrass (Cynodondactylon L. × Cynodontransvaalensis L.) ('Tifway', 'Tifdwarf', 'Tifeagle', 'Kan1') and four common bermudagrass (Cynodon dactylon) ('C299', 'Sportbermuda', 'H10', and 'H19') genotypes were subjected to 14 d of drought stress and detached leaves of two genotypes were exposed to dehydration in growth chambers. Turf quality and leaf relative water content (RWC) decreased while electrolyte leakage (EL) increased during whole-plant drought stress for all genotypes, with more pronounced changes in each parameter for 'C299' and 'Tifeagle' than those for other genotypes ('Tifway', 'Kan 1', 'Sportbermuda', 'H10', and H19'), suggesting that the former two genotypes were more sensitive to drought stress than the other genotypes. During dehydration of detached leaves, relative water loss rate (RWL) was significantly lower in drought-tolerant 'Tifway' than in drought-sensitive 'C299'. Immunoblotting analysis indicated that no dehydrin polypeptides were detected in all genotypes under well-watered conditions. A 24-kDa polypeptide was detected in 'C299' at 6 d of drought, but not in the other genotypes. The dehydrin polypeptides of about 14–74 kDa accumulated at 10 d of drought stress and in a range of RWL for detached leaves, and two dehydrins (31 and 40 kDa) exhibited differential accumulation in the drought-sensitive 'C299' and tolerant 'Tifway', as demonstrated by the whole-plant drought responses. The 31-kDa dehydrin polypeptide was present only in 'Tifway' and 'H19' at 10 d of drought stress, and accumulated with the increasing RWL in detached leaves of 'Tifway'. The expression level of 40-kDa dehydrin polypeptides was greater in 'Tifway'' than in 'C299' at the same level of water deficit (from 10% to 65% RWC). These results indicated that the accumulation of 31- and 40-kDa dehydrins may contribute to drought or dehydration tolerance in warm-season bermudagrass.

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Introduction

Drought is one of the major abiotic factors limiting plant growth. Plants exhibit various responses at both the genomic and proteomic levels during adaptation to drought stress. Dehydrins, a family of hydrophilic proteins with a wide range of molecular masses range from 9 to 200 kDa (Close, 1996), accumulate in many plant species in response to environmental conditions with a dehydrative component, such as drought, cold, and salinity

(Olave-Concha and Bravo, 2005; Beck et al., 2007). These proteins may protect other macromolecules or cellular structures and help in maintaining the integrity of cell membranes (Close et al., 1993; Bray, 1997; Beck et al., 2007). The known physical properties of dehydrins suggest roles as stabilizers of nuclear or cytoplasmic macromolecules under water stress conditions (Campbell and Close, 1997).

Dehydrin proteins have been most extensively studied in relation to drought stress (Labhilili et al., 1995; Close, 1996; Borovskii et al., 2002; Jiang and Huang, 2002). The accumulation of dehydrins was observed in various tissues, such as roots, leaves, coleoptiles, crowns, and seeds (Houde et al., 1992; Close et al., 1993; Han and Kermode, 1996; Mohammadkhani and Heidari, 2008). Dehydrin accumulation has been reported to be positively correlated with dehydration tolerance in annual crops (Close et al.,

1993; Mohammadkhani and Heidari, 2008). For example, drought-tolerant wheat (*Triticum aestivum* L.) plants that exhibited greater shoot dry matter production, a lower reduction in yield and higher leaf water potential under drought stress had stronger expression of 24 kDa dehydrin, compared with drought-sensitive plants (Lopez et al., 2001, 2003). Ozturk et al. (2002) also showed that dehydrin transcripts were up-regulated under drought stress in barley (*Hordeum vulgare* L.). In addition, some studies have reported that an increase in expression of dehydrins is associated with drought tolerance and drought survival in cool-season perennial grasses, such as *Dactylis* and *Poa bulbosa* (Volaire et al., 2001; Volaire, 2002, 2003), and tall fescue (*Festuca arundinacea*) (Jiang and Huang, 2002). Volaire et al. (2001) found that dehydrin proteins of 22, 32, 42, and 44 kDa were expressed in *Dactylis* plants exposed to drought stress, but not detected in well-watered plants. Jiang and Huang (2002) reported that the accumulation of dehydrin proteins (23-, 27-, 40-, 42-, 48-, 53-, and 60 kDa) was induced by progressive water deficit and ABA-treatment in tall fescue (*F. arundinacea* L.). In general, limited information is available on the association of dehydrin expression with leaf desiccation and drought tolerance in perennial grasses. Annual and perennial plants exhibit contrasting responses of dehydrin expression to cope with severe drought stress. Dehydrin accumulation in annual plants appears to be more closely associated with plant tolerance to moderate water deficit rather than for survival of severe drought as found in perennial grasses (Volaire, 2003). The metabolic basis of perennial grass survival of drought stress are likely to be different from those of annual crops in which drought tolerance is evaluated for seed or fruit yield production while drought survival is more important for perennial grasses, particularly under severe stress conditions (Hartung et al., 1998). Therefore, unique dehydrin accumulation in perennial grass species may enhance leaf desiccation tolerance or maintain biomass production, which are desirable traits in perennial grasses used as turf or forage grasses.

Bermudagrass (*Cynodon* spp.) is a widely used warm-season perennial forage and turfgrass and has a wide range of genetic variation in drought tolerance (Taliaferro, 2002). Genetic variation in drought resistance among bermudagrass genotypes has been associated with different mechanisms. Some studies have suggested that drought resistance in some bermudagrass genotypes was associated with lower evapotranspiration, higher root viability, and mass production, deeper roots system and lower root proline content (Carrow, 1995, 1996; Huang et al., 1997a; Qian et al., 1997), while others demonstrated that drought resistance in bermudagrass was correlated with leaf firing and chlorophyll content, RWC, and shoot dry matter production (Huang et al., 1997b). Bermudagrass genotypes contrasting in drought tolerance may exhibit different patterns of dehydrin expression in response to drought stress. The identification of dehydrins associated with drought tolerance may provide useful protein markers for the selection of drought-tolerant perennial grass germplasm.

The objectives of this study were to investigate genetic variation in physiological traits and dehydrin protein accumulation in response to drought stress or dehydration under controlled-environment conditions and to identify dehydrins differentially expressed in bermudagrass genotypes differing in drought tolerance. Two controlled-environment experiments were conducted; one experiment with whole-plant exposure to soil drying for eight genotypes were conducted to evaluate genotypic variation in physiological responses and dehydrin accumulation during progression of soil drying; a detached-leaf experiment was carried out to compare dehydrin expression between two genotypes contrasting in drought tolerance at the same level of water deficit.

Materials and methods

Plant materials and growth conditions

Eight bermudagrass genotypes were used in this study, including four hybrid bermudagrass (*Cynodondactylon* L. × *Cynodontransvaalensis* L.) ('Tifway', 'Tifdwarf', 'Tifeagle', 'Kan1') and four common bermudagrass (*C. dactylon*) ('C299', 'Sportbermuda', 'H10', and 'H19'). Sod plugs of eight genotypes were collected from field plots with 3-year-old grass stand at the turfgrass farm in Shanghai Jiao Tong University, Shanghai, China. Sod pieces were transplanted into polyvinyl chloride pots (22 cm in diameter and 25 cm in height, with holes at the bottom for drainage) filled with a mixture (1:3, v/v) of sand and sandy loamy soil (fine-loamy, mixed mesic Typic Hapludult). Plants were maintained in three growth chambers with a temperature regime of 30/25 °C (day/night), a 14-h photoperiod, 75% relative humidity, and a photosynthetically active radiation of 480 μmol m^{-2} s^{-1} at the canopy level. Plants were irrigated three times per week until soil water reached field capacity or drainage occurred from the bottom of the pot, and fertilized biweekly with controlled-release fertilizers (15 N-15 P$_2$O$_5$-10 K$_2$O) at a total amount of 57 kg ha^{-1}. Turf was hand clipped three times per week at about a 6 cm height. Plants were maintained in the above conditions for 52 d to allow the establishment of turf canopy and root systems.

Treatment and measurements

Two experiments were independently performed. One experiment compared whole-plant responses to drought stress and dehydrin accumulation with progressive drought stress among eight genotypes. The second experiment compared dehydrin accumulation during dehydration of detached leaves between two genotypes contrasting in drought tolerance ('Tifway' and 'C299').

Experiment I: whole-plant responses to drought stress

The whole-plant experiment consisted of two water treatments (well-watered control and drought stress) imposed on eight genotypes. Drought stress was imposed by withholding irrigation for 14 d until complete wilting of full lamina was observed in some plants. For the well-watered control, plants were watered every other day to completely saturate the soil in each container (until water emerged from the bottom of the container). Four replicates of the control and drought-stressed plants for each of the eight genotypes were randomly placed in three growth chambers. All containers in three growth chambers were relocated every 2 d inside the chamber and between chambers to minimize environmental variations within and among the chambers.

The variation in drought tolerance of whole-plants among eight genotypes was evaluated by rating turfgrass quality and measuring cell membrane stability and leaf relative water content (RWC). Turf quality was rated visually based on turfgrass color (extent of chlorosis and leaf senescence), plant density, and degree of leaf wilting on a 1–9 scale (1 = brown, senesced, and desiccated turf and 9 = fully turgid, green, and dense turf). RWC was determined using 10–15 whole fully-expanded leaves or full lamina per container according to Barrs and Weatherley (1962). Leaf samples were detached from the plants and immediately weighed to determine fresh weight (FW). Samples were placed into covered Petri dishes filled with water for leaves to reach full hydration. After approximately 24 h at 4 °C, leaf samples were blotted dry with paper towels, and weighed to determine turgid

weight (TW). Leaf tissue was then dried in an oven at 80 °C for 48 h to determine dry weight (DW). Leaf RWC was calculated as $(FW-DW)/(TW-DW) \times 100$.

Cell membrane stability was determined as electrolyte leakage (EL). For EL analysis, whole fully-expanded leaves (0.1 g from each pot) were cut to segments (approximately 0.5 cm long) were incubated in 15 mL distilled deionized water on a shaker for 24 h. The conductance of the incubation solution was measured as the initial level of EL (C_i) using a conductance meter (YSI Model 32, YSI Incorporated, Yellow Springs, OH, USA). Leaf tissue in the incubation solution was killed in an autoclave at 120 °C for 30 min. The conductance of the incubation solution with killed tissues (C_{max}) was determined following 24-h incubation on a shaker. Relative EL was calculated as $(C_i/C_{max}) \times 100$.

Immunoblotting analysis of dehydrins

Immunoblotting analysis of dehydrin was performed on plants exposed to drought stress at 6 and 10 d of drought for all genotypes. Total soluble protein was extracted from leaves according to the method of Li et al. (1996) and Patton et al. (2007) with modifications. Leaf tissue (0.5 g FW) was ground to powder using pre-chilled mortar and pestle in liquid nitrogen to a fine powder. Protein was extracted in 4 mL of ice-cold phosphate buffer (150 mM, pH 7.0). Solutions were then centrifuged at 14000g at 4 °C for 40 min, and supernatant was collected for protein analysis. Proteins were quantified by the dye-binding assay (Bradford, 1976). An equal amount of proteins (30 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with a JY-SCZ-2 electrophoresis unit (Jun Yi Oriental, Beijing, China) using a 5% stacking gel and 12% running gel. Separated polypeptides were transferred onto a polyvinylidene fluoride (PVDF) membrane (IPVH00010, Millipore, USA) with a VE-186 electrophoresis transfer unit (Tian Neng, Shanghai, China) using a continuous buffer system [0.39 M glycine, 0.48 M Tris, 20% methanol(v/v)] at 100 V for 1 h. The membranes were then incubated with a 1:2000 dilution of rabbit anti-dehydrin polyclonal antibody (PLA-100, Stressgen Biotechnologies Corporation, Victoria, BC, Canada) prepared against a synthetic peptide containing the conserved sequence EKKGIMDKIKELPG (Close et al., 1993) in Tris-Buffered Saline Tween-20 (TBST) for 1 h after blocked in 5% non-fat milk solution overnight at 4 °C. Goat anti-rabbit IgG antibody (dilution 1:4000) conjugated to alkaline phosphatase (A0239, Beyotime Institute of Biotechnology, China) was used as the second antibody and was detected by 5-bromo-4-chromo-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT) substrate (Blake et al., 1984). Immunoblotting was repeated using three separate samples for each genotype and each sample was repeated in three gels. A representative blotting image for each treatment was present in Fig. 4. The intensity of protein bands was analyzed for optical intensity using a protein imaging software (Tanon 2500, Tanon Science and Technology Co., Ltd., Shanghai, China).

Experiment II: dehydration of detached leaves

In the dehydration experiment, detached leaves of drought-tolerant 'Tifway' and drought-sensitive 'C299' were allowed to dry to different levels of water deficit. Fresh, fully-expanded leaves (0.8 g) were detached from plants, weighed immediately for FW, and then placed in Petri dishes (15 mm in diameter) in a growth chamber under constant temperature (25±0.5 °C), light intensity (150 μmol m^{-2} s^{-1}) and relative humidity (50%±3%). Water loss from the detached leaves was measured by weighing the samples at 20, 40 60, 90, 120, 150, 180, 210, 240, 300, 360, and 420 min

after the initial weighing. The dehydration experiment was repeated four times, with four replicated samples at repetition. RWL in detached leaves was monitored using the technique described by Anderson (1984) and Ristic and Jenks (2002) with modification. RWL was calculated using the following equations: RWL (mg g^{-1} DW min^{-1}) = (FW−Wt)/(DW × T) or RWL (%) = [(FW−Wt)/FW] × 100, where FW is the initial FW of the sample after detached from plants, Wt is the FW of the sample after each dehydration period, DW is the DW of leaves after drying in an oven at 80 °C for 48 h, and T (min) is the duration of dehydration. When RWL (%) reached to the predetermined rates at 10%, 30%, 50%, and 65%, leaf samples were placed in liquid nitrogen and stored for gel blotting of dehydrin expression following the procedure described above. Immunoblotting was repeated using three separate samples for each genotype and each sample was repeated in three gels. A representative blotting image for each treatment was present in Fig. 5. The intensity of protein bands was analyzed for optical intensity using a protein imaging software (Tanon 2500, Tanon Science and Technology Co., Ltd., Shanghai, China).

Statistical analysis

The data in both experiments were analyzed using the analysis of variance to determine the effects of water or dehydration treatments, genotypes, and their interactions. The differences among genotypes and among treatments for a given genotype were separated using Fisher's protected least significant difference (LSD) test at the $P = 0.05$ level of probability (SAS Institute, 1988).
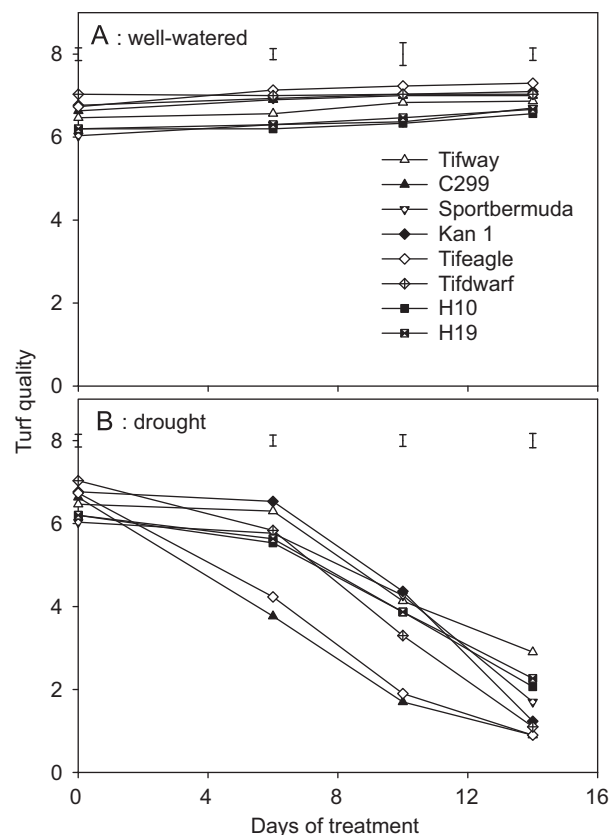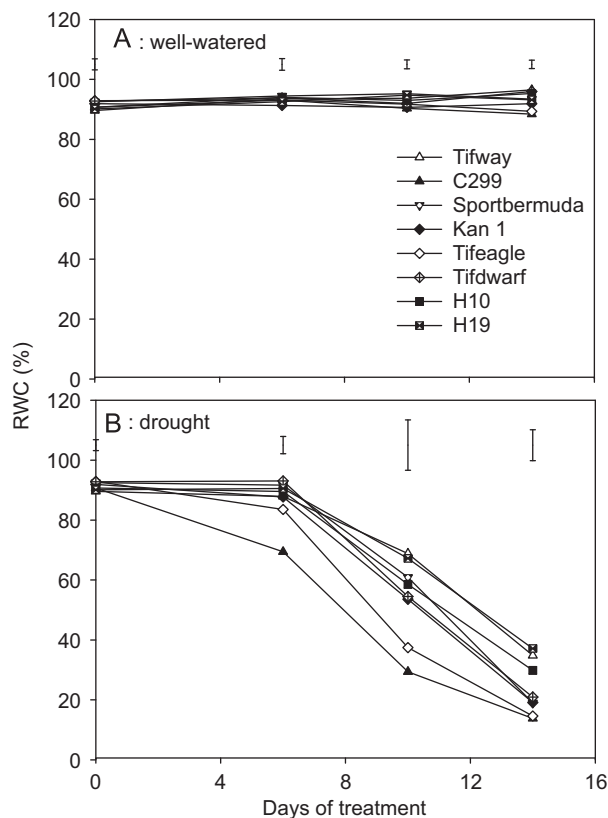

Fig. 1. Variation in turf quality for eight genotypes of bermudagrass under well-watered (A) and drought stress (B). Vertical bars on the top indicate LSD values ($P = 0.05$) for the comparison between genotypes at a given day of treatment.

Fig. 2. Variation in leaf relative water content (RWC) for eight genotypes of bermudagrass under well-watered (A) and drought stress (B). Vertical bars on the top indicate LSD values ($P = 0.05$) for the comparison between genotypes at a given day of treatment.

Fig. 3. Variation in leaf electrolyte leakage (EL) for eight genotypes of bermudagrass under well-watered (A) and drought stress (B). Vertical bars on the top indicate LSD values ($P = 0.05$) for the comparison between genotypes at a given day of treatment.
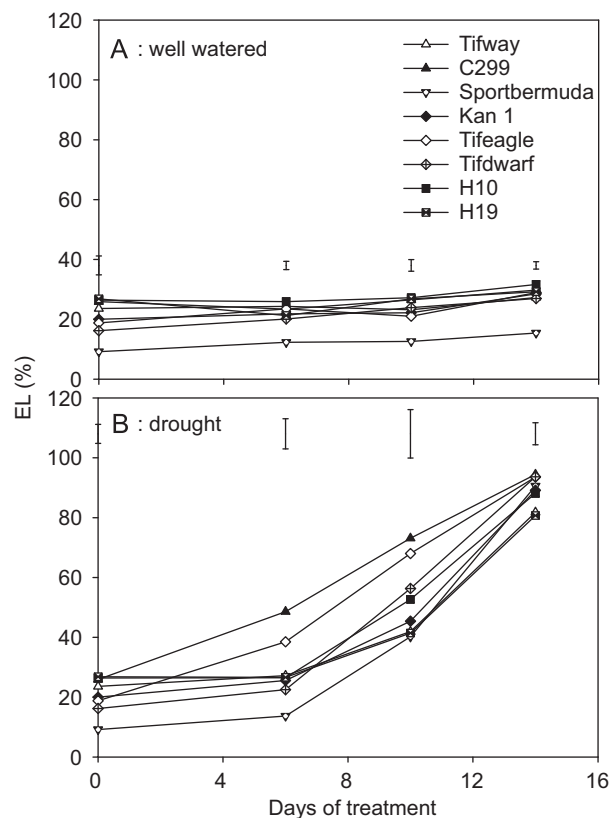
Results

Whole-plant physiological responses to drought stress in eight genotypes

Turf quality and leaf relative water content (RWC) of all genotypes were maintained at the same level (no statistical difference) under well-watered conditions (Figs. 1A and 2A), but exhibited rapid decline during drought stress (Figs. 1B and 2B). Turf quality and RWC in 'Tifeagle' and 'C299' exhibited more dramatic decline than in other genotypes during drought stress. 'Tifway' and 'H19' had significantly higher turf quality (Fig. 1B) and RWC (Fig. 2B) than in 'Tifeagle' and 'C299' at 6, 10, and 14 d of treatment (Fig. 1B) while other genotypes ('Sportbermuda', 'Kan 1', and 'H10') had higher turf quality than 'Tifeagle' and 'C299' at 6, 10, and 14 d of stress, and had higher RWC than 'Tifeagle' and 'C299' only during the first 10 d of drought stress.

Leaf electrolyte leakage (EL) of all genotypes, except for 'Sportbermuda', was maintained at the same level and did not change significantly under well-watered conditions (Fig. 3A). The level of EL increased in all genotypes and genotypes during drought stress, but the rate of increase varied with genotype (Fig. 3B). Leaf EL was significantly higher in 'C299' and 'Tifeagle' than in 'Sportbermuda', 'Kan 1', and 'H10' within 10 d of drought stress, and than in 'Tifway' and 'H19'during the entire 14-d drought treatment.

Dehydrin expression of eight genotypes expression to drought stress

No dehydrin polypeptides were detected in fully hydrated leaves of well-watered plants (Fig. 4A). Immunoblot analysis with anti-dehydrin polyclonal antibodies detected one dehydrin polypeptide (24 kDa) in 'C299' but not in other cultivars at 6 d of drought (Fig. 4B). At 10 d of drought stress, six dehydrin polypeptides of 74, 40, 31, 24, 19, and 14 kDa were detected in eight genotypes (Fig. 4C). The 74-, 19-, and 14-kDa polypeptides accumulated in all genotypes, and no genotypic differences were observed in the level of expression. The 40-kDa polypeptides had lower expression level in 'C299', 'Tifdwarf', and 'Tifeagle', which was 7–10% lower than the other five genotypes (Fig. 4C, lanes 4, 5, and 8). The 31-kDa dehydrin polypeptide was detected only in 'H19' and 'Tifway' (Fig. 4C, lanes 1 and 8), but was not expressed in the other six genotypes. The 24-kDa polypeptides expressed in all genotypes, but the intensity in 'Tifdwarf', 'Tifeagle', and 'C299' (Fig. 4C, lanes 4, 5, and 8) were higher than in the other genotypes, and the level of expression was 16–21% higher than in the other genotypes.
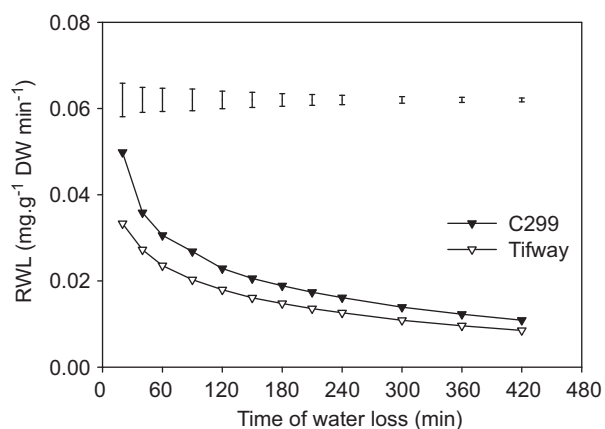
Water loss rate during dehydration of detached leaves

'Tifway' had a slower RWL than 'C299' during dehydration, with the greatest difference in RWL between the two genotypes observed within the first 20 min of dehydration (Fig. 5). The difference in RWL between the two genotypes, however, became smaller following longer periods of dehydration up to 7 h.

The time for detached leaves to lose water by 10%, 30%, 50%, and 65% varied between 'Tifway' and 'C299'. For 'C299', RWL reached 10%, 30%, 50 %, and 65% after leaves were dehydrated for 10, 65, 160 and 300 min, respectively, whereas RWL were 10%, 30%, 50%, and 65% after 15, 75, 180, and 360 min of dehydration, respectively, for 'Tifway'.
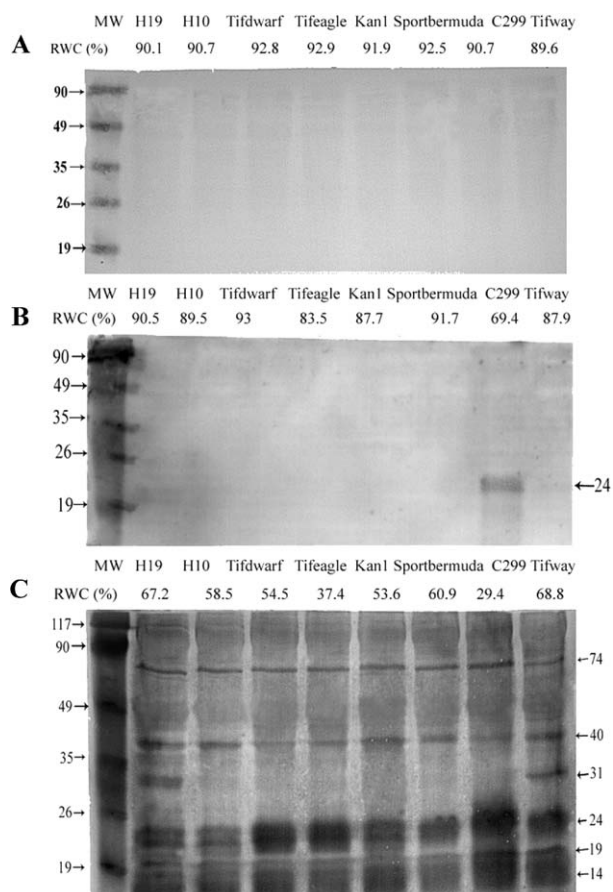
Fig. 4. Immunoblots of dehydrin expression in bermudagrass genotypes during drought stress. (A, well-watered control; B, 6 d of drought; C, 10 d of drought; From left to right: Markers; lane 1, H19; lane 2, H10; lane 3, Tifdwarf; lane 4, Tifeagle; lane 5, Kan 1; lane 6, Sportbermuda; lane 7, C299; lane 8, Tifway).

Fig. 5. Rate of water loss in detached leaves of 'Tifway' and 'C299' during dehydration. Vertical bars on the top indicate LSD values ($P = 0.05$) for the comparison between genotypes at a given day of treatment.

### Dehydrin accumulation during dehydration of detached leaves

Immunoblots of dehydrin proteins in detached leaves of 'Tifway' and 'C299' with RWL of 2%, 10%, 30%, 50%, and 65% were performed to examine changes in dehydrin accumulation with different levels of water loss or water deficit and compare difference in dehydrin expression under the same level of water deficit between the two genotypes (Fig. 6). The immunoblot analysis detected the accumulation of 74-, 40-, 31, 24-, 19-, and 14 kDa dehydrin polypeptides across the RWL range in both genotypes, but no significant difference was observed in 2% of RWL. The intensity of 74-kDa dehydrin polypeptides was very low in 2% of RWL, and did not change with increasing RWL from 10% to 65% in both 'Tifway' and 'C299'. With the increase in RWL, the intensity of 40-kDa dehydrin proteins was elevated. The expression level was increased by 112%, 124%, 135%, and 167% at 10%, 30%, 50%, and 65% of RWL in 'C299', and increased by 124%, 155%, 172%, and 236% at 10%, 30%, 50%, and 65% of RWL in 'Tifway' compared with 2% of RWL, respectively. The accumulation of 40-kDa dehydrin polypeptides in 'Tifway' was more pronounced than in 'C299' at the same level of water deficit (RWL from 10% to 65%), which was 7%, 16%, 18%, and 28% higher than in 'C299'. The accumulation of 31-kDa dehydrin was detected in 'Tifway', but not in 'C299' during dehydration. As RWL increased from 10% to 65%, the intensity of 31-kDa dehydrin increased in 'Tifway', which increased by 12%, 18%, and 108% compared with 10% of RWL. The intensity of 24-, 19-, and 14-kDa dehydrins increased with RWL from 10% to 65% for both genotypes, but to a greater extent for 'C299', and the expression level of dehydrin in 'C299' was 5–7% higher than in 'Tifway' at 10%, 30%, 50%, and 65% of RWL.

Discussion

Drought-induced accumulation of dehydrin proteins has been associated with drought tolerance in many plant species, especially annual crops (Close et al., 1993; Lopez et al., 2001, 2002, 2003; Mohammadkhani and Heidari, 2008). In this study, six dehydrin polypeptides were detected in bermudagrass genotypes under drought stress conditions. However, the type or size of dehydrins and the level of accumulation changed with water availability or level of water deficit and varied among genotypes differing in drought tolerance, as demonstrated in both the whole-plant and detached-leaf experiments.

The accumulation of 40-kDa dehydrin polypeptides increased with progressive water deficit when plant RWL increased from 10% to 65% in detached leaves of 'Tifway' and 'C299', and the expression level was higher in 'Tifway' than in 'C299' at the same level of water deficit. The 31-kDa dehydrins were exclusively expressed in 'Tifway' and 'H19' at 10 d of drought stress even when leaf RWC was significantly higher in 'Tifway' and 'H19' than in the other six genotypes. Furthermore, the intensity of 31-kDa dehydrins increased with RWL ranging from 10% to 65% during dehydration of detached leaves. Under the same level of water deficit, the expression of 31-kDa dehydrins was stronger in 'Tifway' leaves compared with that in 'C299', suggesting that the accumulation of these dehydrins was positively associated with water deficit tolerance in bermudagrass. Similar size dehydrins were also observed in leaves of several other cool-season perennial grass species. Dehydrins of 31-kDa were detected in leaves of tall fescue induced by drought and ABA-treatment (Jiang and Huang, 2002). Olave-Concha et al. (2004) reported that the accumulation of 30-, 32-, and 42 kDa dehydrin proteins induced by ABA, dehydration, NaCl, and low osmotic potential may play a role in stress injury prevention in leaves of *Deschampsia antarctica*. Volaire et al. (2001) reported that high levels of accumulation of 32-, 41-, and 42 kDa dehydrin polypeptides in leaves *P. bulbosa* was associated with dehydration tolerance. However, to our knowledge the 30 kDa family of dehydrins have been found mainly in seeds or embryos of annual plants, which has been associated with seed dormancy or seed desiccation tolerance (Wechsberg et al., 1994; Kermode, 1997), but was not previously
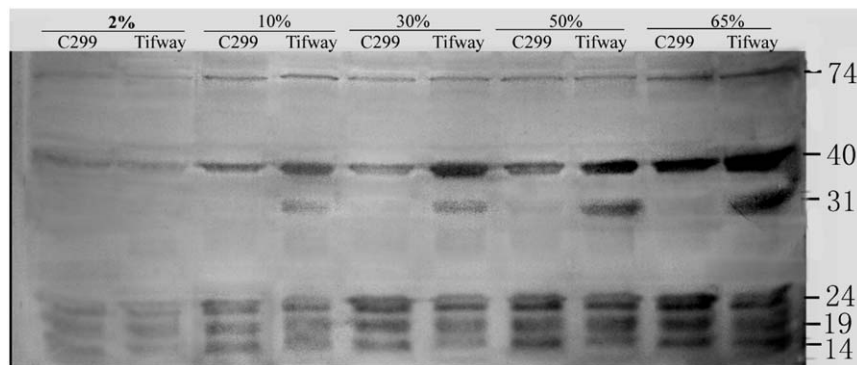
Fig. 6. Immunoblots of dehydrins expression in detached leaves of 'Tifway' and 'C299' at different rate of water loss (RWL %). lane 1, C299 2%; lane 2, Tifway 2%; lane 3, C299 10%; lane 4, Tifway 10%; lane 5, C299 30%; lane 6, Tifway 30%; lane 7, C299 50%; lane 8, Tifway 50%; lane 9, C299 65%; lane 10, Tifway 65%.
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reported in leaves of annual plants. It appeared that this family of dehydrins may be specific for perennial plant responses to drought stress, particularly associated with leaf dehydration tolerance. However, the specific functions of these dehydrins involved in drought adaptation are still unclear, despite findings of previous studies that suggest the accumulation of dehydrins in response to water deficit may help control water loss through osmotic protection of cells from further dehydration during drought stress (Close, 1996; Han and Kermode, 1996; Cellier et al., 1998; Volaire, 2002).

The accumulation of 74-kDa dehydrin polypeptides did not differ among eight bermudagrass genotypes in the whole-plant drought experiment and also expressed in detached leaves of 'Tifway' and 'C299' across the range of RWL from 2% to 65% water deficit. Volaire et al. (2001) have reported a similar size dehydrin protein (approximately 78 kDa) was expressed in both irrigated leaf tissues and drought-stressed tissues in *P. bulbosa* L. Our study suggests that 74-kDa dehydrin proteins were constitutively expressed and not regulated by drought stress, and thus their expression was not related to drought tolerance.

Three low molecular weight dehydrins (14, 19, and 24 kDa) were accumulated in all eight bermudagrasses at 10 d of drought stress or in detached leaves of 'Tifway' and 'C299' with increasing accumulation from 2% to 65% RWL. The 19-, and 24-kDa dehydrin proteins have been previously reported in *P. bulbosa*, a cool-season perennial grass, especially present in the dormant (under both drought and irrigated conditions) and drought-resistant genotypes, which suggested that the accumulation of these low molecular weight dehydrins have a major role in summer dormancy and drought survival related to drought avoidance (Volaire, 2002; Volaire et al., 2005). The 14-kDa dehydrin proteins have also been found in other species (Muthalif and Rowland, 1994; Arora et al., 1997). The dehydrins in blueberry (*Vaccinium corymbosum*) leaves were strongly induced by cold stress, but to a lesser extent by drought stress and their accumulation not closely related to changes in leaf water status during drought stress (Panta et al., 2001). When compared at the same level of water loss, the expression level of these smaller molecular weight dehydrins was higher in 'C299' than in 'Tifway'. These results suggested that the accumulation of 14-, 19-, and 24-kDa dehydrins was a response to water loss during progressive drought stress rather than an adaptive mechanism to drought stress. Different types or size of dehydrins polypeptides could be induced by different stresses and the same size of dehydrin expressed in different species exhibit distinct functions (Rorat, 2006). Previous research in cool-season perennial grass species suggest that low molecular weight dehydrin proteins was correlated to drought survival and summer dormancy, while our results demonstrated that these proteins was only a response to water deficit in warm-season bermudagrass.

In summary, bermudagrass genotypes exhibited significant genetic variation in drought tolerance. In the whole-plant responses to drought stress experiment, 'Tifway' and 'H19' performed the best, 'C299' was the most drought-sensitive, and other genotypes were intermediate in drought performance, based on the data in turf quality, RWC, and EL. The dehydration experiment with the detached leaves further confirmed the difference between 'Tifway' and 'C299', as a tolerant and sensitive genotype, respectively. The immunoblot analysis detected different sizes of dehydrin polypeptides, and the accumulation of 31- and 40 kDa dehydrin proteins was positively associated with drought tolerance or water deficit tolerance in bermudagrass genotypes. These dehydrins represent potential protein markers for the selection of warm-season turfgrass genotypes with enhanced drought tolerance.

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