Drought Survival and Recuperative Ability of Bentgrass Species Associated with Changes in Abscisic Acid and Cytokinin Production

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ABSTRACT. Abscisic acid (ABA) and cytokinins are two groups of plant hormones that play important roles in regulating plant responses to decreases in soil water availability. The primary objective for this study was to determine whether species variability in drought survival and recovery for colonial bentgrass (*Agrostis capillaris* L.), creeping bentgrass (*A. stolonifera* L.), and velvet bentgrass (*A. canina* L.) were related to changes in ABA and cytokinin content. Plants of 'Tiger II' colonial bentgrass, 'L-93' creeping bentgrass, and 'Greenwich' velvet bentgrass were subjected to two soil moisture treatments: 1) well-watered controls, irrigated three times per week; and 2) drought, irrigation completely withheld for 16 days. For recovery, previously drought-stressed plants were rewatered and irrigated three times per week to evaluate the recovery potential for each species. Drought stress resulted in significant declines in turf quality (TQ), shoot extension rates, canopy net photosynthetic rate (Pn), daily evapotranspiration rate (ET), and cytokinin content, and significant increases in ABA content for all three bentgrass species. Velvet bentgrass exhibited less severe drought injury, as exhibited by higher TQ, Pn, and daily ET rate compared with colonial bentgrass and creeping bentgrass. Velvet bentgrass also had significantly less ABA accumulation, which could allow for continued gas exchange and sustained plant survival during drought stress compared with colonial bentgrass and creeping bentgrass. Upon rewatering after drought stress, colonial bentgrass exhibited more rapid recovery in turfgrass growth and water use compared with creeping bentgrass and velvet bentgrass. The higher recuperative ability of colonial bentgrass could be associated with its more rapid decline in ABA content and increases in cytokinin content compared with creeping bentgrass and velvet bentgrass.

Plant hormones are naturally occurring substances that, in small concentrations, can exert significant changes in plant growth and developmental processes. Changes in hormone concentration or hormone balance could significantly affect the responses of plants to environmental stresses. Abscisic acid (ABA) and cytokinins are two major groups of plant hormones that play important roles in regulating plant responses to decreases in soil water availability (Pospíšilová et al., 2000; Wilkinson and Davies, 2002). The involvement of these hormones in root-to-shoot signaling, particularly through regulation of stomatal behavior and leaf growth, has been implicated in plant resistance to drought (Pospíšilová et al., 2000; Quarrie, 1989, 1993).

Recent studies have confirmed that ABA is the primary chemical signal moving from roots to shoots in response to soil drying, resulting in stomatal closure and an overall decrease in water loss via transpiration (Assmann and Shimazaki, 1999; Davies et al., 2002; Wilkinson and Davies, 2002; Zhang and Davies, 1989). Abscisic acid may also be involved in plant adaptation to drought stress by inhibiting leaf growth or

transpirational area (Alves and Setter, 2000; Bacon et al., 1998) and inducing the production of antioxidant enzymes (Bellaire et al., 2000; Bueno et al., 1998; Jiang and Zhang, 2002) and upregulating genes related to drought resistance (Bray et al., 1999; Campbell et al., 2001; Jin et al., 2000; Tamminen et al., 2001).

Some species with high leaf ABA accumulation have been reported to be more drought tolerant than those with low ABA accumulation (Henson et al., 1981; Larque–Saavedra and Wain, 1976). In contrast, low ABA accumulation has been positively related to drought resistance, such as in orchardgrass (*Dactylis glomerata* L.) (Volaire et al., 1998), wheat (*Triticum aestivum* L.) (Innes et al., 1984; Quarrie, 1989), maize (*Zea mays* L.) (Ilahi and Dorffling, 1982), and sorghum [*Sorghum bicolor* (L.) Moench] (Durley et al., 1983). Wang et al. (2003) reported that Kentucky bluegrass (*Poa pratensis* L.) cultivars tolerant of drought exhibited lower ABA accumulation rates than drought-sensitive cultivars during short-term drought stress, suggesting that a low accumulation rate of ABA in leaves would be beneficial for the maintenance of photosynthesis during short-term drought.

Cytokinins generally have antagonistic physiological effects with ABA, especially in stomatal control. Cytokinins maintain stomatal opening, and thus increase stomatal conductance and transpiration rates (Blackman and Davies, 1985; Incoll and Jewer, 1987; Jewer and Incoll, 1980; Lechowski, 1997). Cytokinins also delay leaf senescence (Čatský et al., 1996; Naqvi, 1999) and enhance photosynthetic protein synthesis and electron transport (Synková et al., 1997). Application of cytokinin-containing extracts increased leaf zeatin riboside (ZR) content of creeping bentgrass, which resulted in increased

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turf quality (TQ) under drought stress (Zhang and Ervin, 2004). Increased levels of cytokinins have also been associated with improved heat stress for creeping bentgrass (Liu et al., 2002).

There is considerably less information on the effects of cytokinins alone or their interaction with ABA during water deficit compared with what is known of the relationship between ABA and the drought stress response. This has been attributed to the great variability in plant cytokinin compounds as well as lower concentration of cytokinins in plants compared with ABA (Beck, 1996; Hansen and Dorffing, 2003). In general, though, there is decreased cytokinin accumulation in drought-stressed plants, which can amplify responses of shoots to increasing ABA levels (Goicoechea et al., 1995, 1997; Naqvi, 1995). The ratio of ABA to cytokinin may be important for regulation of gas exchange and water relations rather than absolute changes in concentration of either hormone (Cheikh and Jones, 1994; Goicoechea et al., 1997; Moore-Gordon et al., 1998). A decline in ABA content along with increases in cytokinin content have been reported to play important roles in facilitating plant recovery from drought stress in some plant species (Ivanova et al., 1998; Yordanov et al., 1997, 1999).

Limited work in turfgrasses has demonstrated differences in ABA accumulation and sensitivity among genotypes differing in drought tolerance, and even less information is available on changes in cytokinins associated with drought survival and recovery for turfgrasses (Henson and Turner, 1991; Pekic et al., 1995; Wang and Huang, 2003; Wang et al., 2003). Understanding hormonal changes associated with turfgrass tolerance to drought stress may provide further insight into drought survival mechanisms for turfgrasses growing in water-limiting environments. Therefore, the objectives of this study were to examine whether bentgrass species variability in drought survival were related to changes in ABA and cytokinin accumulation under drought stress and recovery from drought stress.

Materials and Methods

PLANT MATERIALS AND GROWTH CONDITIONS. Sods of 'Tiger II' colonial bentgrass, 'L-93' creeping bentgrass, and 'Greenwich' velvet bentgrass were collected from field plots at the Rutgers Turfgrass Horticulture Farm II in North Brunswick, N.J., and were transplanted into PVC tubes (10 cm in diameter, 40 cm long) filled with a 1:3 (v/v) sterilized mixture of sand and sandy loam (fine-loamy, mixed, mesic, Typic Hapludult). A nylon screen covered the bottom of each PVC tube to contain the soil and allow for drainage of water from the tubes.

Plants were grown in a greenhouse to allow for plant establishment. Turf was irrigated five times weekly to maintain plants under well-watered conditions, and were hand clipped weekly at ≈3-cm height. Half-strength Hoagland's solution (Hoagland and Arnon, 1950) was applied weekly to provide nutrients and to facilitate plant establishment before initiation of treatments. After ≈60 d of establishment in the greenhouse, plants were transferred to a growth chamber (20 °C/15 °C day/night temperatures, 14-h photoperiod, relative humidity of 55% to 65%, and a photosynthetic photon flux density of 600 μmol·m^{-2}·s^{-1}) and allowed to acclimate for 14 d before treatments were imposed.

TREATMENTS. The experiment consisted of two treatments: 1) well-watered control, maintained at ≈31% soil water content (v/v) and 98% leaf relative water content throughout the study period; and 2) drought-recovery, irrigation completely withheld for 16 d then rewatered to allow for recovery for 34 d. At the end of the drought period (16 d), a majority of plants were desiccated and brown, which corresponded to a soil water content of 11% (v/v) and leaf relative water content of 38% for all three bentgrass species. Soil water content was assessed at the 0 to 20-cm soil depth using the time-domain reflectometry method (Topp et al., 1980) with three-pronged waveguides installed vertically in the 0 to 20-cm soil layer (Soil Moisture Equipment, Santa Barbara, Calif.). Leaf relative water content was determined using 10 to 15 fully expanded leaves per pot according to Barrs and Weatherley (1962).

MEASUREMENTS. The effects of drought stress on turfgrass performance were evaluated by examining changes in TQ, shoot extension rates, and gas exchange at different times of treatment. TQ was rated based on color (extent of chlorosis and leaf senescence), plant density, and degree of leaf wilting on a scale of 1 to 9 points (1 point, brown, senesced, and desiccated turf; 9 points, fully turgid, green, and dense turf). A quality rating of 6 points and higher on the scale indicated acceptable levels (mostly green or nonchlorotic leaf tissue and little leaf wilting). Plant recovery after drought stress was evaluated as a percentage of green and turgid leaf tissue of the plant canopy. Shoot extension rate was measured every 5 d during the drought treatment by measuring the difference in canopy height at 5-d intervals.

Canopy net photosynthetic rate (Pn) and evapotranspiration (ET) rates were measured ≈5 h into the 14-h photoperiod using an infrared gas exchange analyzer (LI-COR 6400; LI-COR, Inc., Lincoln, Nebr.) with a custom-designed acrylic canopy chamber supplied with 400 μL·L^{-1} CO$_2$. The canopy chamber (10-cm diameter, 10-cm height) was attached to a PVC joining collar that fit tightly over the PVC tubes containing the plants. The top of the canopy chamber was covered with transparent Propa-film (LI-COR, Inc.) for light penetration during gas exchange measurements. Canopy gas exchange parameters were measured at 5, 10, 13, and 16 d of drought treatment, and at 5, 10, and 17 d of recovery after drought treatment. Canopy transpiration is reported as ET because canopy measurements of turfgrass transpiration also may include evaporation from the soil surface (Kneebone et al., 1992).

On the same day that gas exchange measurements were acquired, ≈0.3 g of leaves were randomly sampled for hormone analysis from each pot. Leaves were excised and wrapped in aluminum foil, and then immediately submerged into liquid nitrogen. Samples were stored at –80 °C until extraction of leaf hormones. The most commonly occurring cytokinins are isopentyl adenosine (iPA), trans-zeatin (Z)/ZR, and dihydroxy-zeatin/dihydroxyzeatin riboside. However, because of the extent of sample required for analysis of ABA and the three cytokinins, iPA content was determined because it has been demonstrated to be the predominant form of cytokinins in leaves of bentgrass species (Liu and Huang, 2005; Wang et al., 2004). Abscisic acid and iPA extraction, purification, and quantification were performed according to the method described previously by Setter et al. (2001) and modified by Wang et al. (2003). Briefly, leaves were extracted in 80% (v/v) methanol with 1% glacial acetic acid (v/v) and 10 mg·L^{-1} butylated hydroxytoluene using a weight per volume of 1 sample : 10 extraction solution. Supernatants were vacuum dried and resuspended in 10 mM triethyl amine, and then aliquots were applied onto C$_{18}$ chromatography columns

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(Supelco, Bellefonte, Pa.) for purification of the plant hormones. The ABA- and iPA-containing fractions were eluted with 55% and 30% methanol respectively, vacuum dried, and redissolved with Tris-buffered saline solution (pH, 7.5). Abscisic acid and iPA were quantified using monoclonal antibodies (Agdia, Inc., Elkhart, Ind.) by indirect enzyme-linked immunosorbent assay. Final quantification of ABA and iPA were determined based on the absorbance at 405 nm using an automated microplate reader (EL800; Bio-Tek Instruments, Winooski, Vt.) and compared with corresponding standard curves for each of the hormones.

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS. The experiment was a completely randomized design with five replicates for a total of 30 plants. The plants were rotated weekly to avoid any potential edge effects of the growth chamber. Effects of water treatment, species, and corresponding interactions were determined by analysis of variance according to the general linear model procedure of SAS (version 8.2; SAS Institute, Cary, N.C.). Differences between treatment means were separated by the LSD test at a *P* value of 0.05.

Results

Turf quality declined with drought duration for all three bentgrass species (Fig. 1), whereas TQ for well-watered controls was maintained between 8 and 9 points throughout the experimental period regardless of species (data not shown). At 9 d of drought treatment, TQ for velvet bentgrass (7.5 points) was significantly greater than that of creeping bentgrass (6.0 points); colonial bentgrass exhibited intermediate TQ among the three species at this duration of drought treatment (6.5 points). By 12 d of treatment, TQ for both colonial bentgrass and creeping bentgrass significantly declined below the minimal acceptable quality level (6.0 points), whereas velvet bentgrass had a TQ rating of 6.5 points. By the end of the drought treatment, there were no significant differences in TQ among the three species, with TQ declining to 1.3 points for colonial bentgrass and creeping bentgrass, and 2.3 points for velvet bentgrass.

After 5 d of rewatering, colonial bentgrass exhibited ≈40% recovery based on the extent of green, turgid leaf tissue in the

Fig. 2. Recovery of TQ canopy parameters, expressed as a percentage of green and turgid leaves, for colonial bentgrass, creeping bentgrass, and velvet bentgrass during rewatering after drought stress. Treatment means for each species are the average of five measurements (replicates). Vertical bars are LSD values (*P* ≤ 0.05) indicating statistically significant differences for species comparisons at a given day of treatment.

plant canopy (Fig. 2). This was significantly higher than the recovery observed for creeping bentgrass and velvet bentgrass, which exhibited 22% and 28% recovery respectively. At 10 d of rewatering, more than 50% recovery was observed for all three species, ranging from 52% for velvet bentgrass to 65% for colonial bentgrass. There were no significant differences for recovery among the bentgrass species at 10 and 14 d of recovery. However, by 17 d of treatment, colonial bentgrass and creeping bentgrass exhibited higher levels of recovery (88% and 89% respectively) compared with velvet bentgrass (77%). At 34 d of rewatering, all three bentgrasses exhibited ≈100% recovery.

During the first 5 d of drought stress, the average shoot extension rate was the same between well-watered and drought-stressed plants of colonial bentgrass, creeping bentgrass, and velvet bentgrass (Fig. 3). Under well-watered conditions, shoot extension rates did not differ (*P* > 0.05) between plants of colonial bentgrass, creeping bentgrass, and velvet bentgrass. Shoot extension rates were 45%, 65%, and 60% lower compared with well-watered controls during 6 to 10 d of drought treatment for colonial bentgrass, creeping bentgrass, and velvet bentgrass respectively. At this time, colonial bentgrass had the highest average shoot extension rate, and creeping bentgrass the lowest extension rate; velvet bentgrass exhibited intermediate shoot extension among the three species. By 11 to 15 d of drought treatment, the shoot extension rate declined to between 0.02 and 0.05 cm/day. There were no differences (*P* > 0.05) in shoot extension rate among the three species at this duration of treatment.

Canopy net photosynthetic rate and ET are presented as a percentage of the prestress (day 0), well-watered levels for each species (Figs. 4 and 5). Well-watered control plants for all three species maintained Pn and ET levels at prestress levels throughout the treatment period (data not shown). At 10 d of drought stress, Pn declined to 0% and 36% of prestress levels for creeping bentgrass and colonial bentgrass respectively, whereas no significant decline in Pn was observed for velvet bentgrass. By 13 d of treatment, Pn for colonial bentgrass and creeping bentgrass dropped to 0%, whereas Pn for velvet bentgrass was 55% of prestress levels. Canopy net photosynthetic rate was 0% for all three species at 16 d of drought stress. During the drought treatment, velvet bentgrass maintained
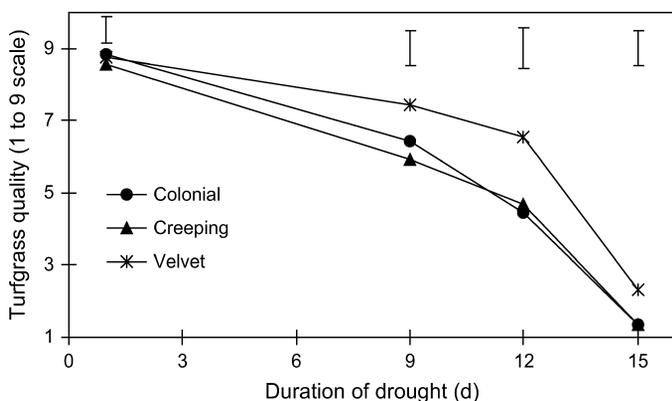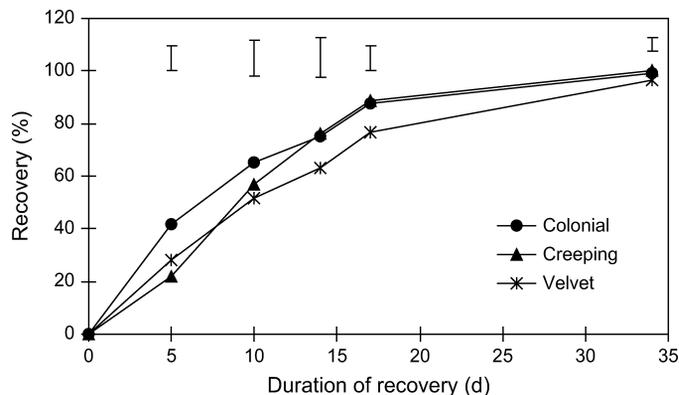

Fig. 1. Changes in TQ in response to drought stress for colonial bentgrass, creeping bentgrass, and velvet bentgrass. A value of 9 points represents the best TQ (green, dense canopy) and 1 point represents the lowest TQ (brown, desiccated). Treatment means for each species are the average of five measurements (replicates). Vertical bars are LSD values (*P* ≤ 0.05) indicating statistically significant differences for species comparisons at a given day of treatment.
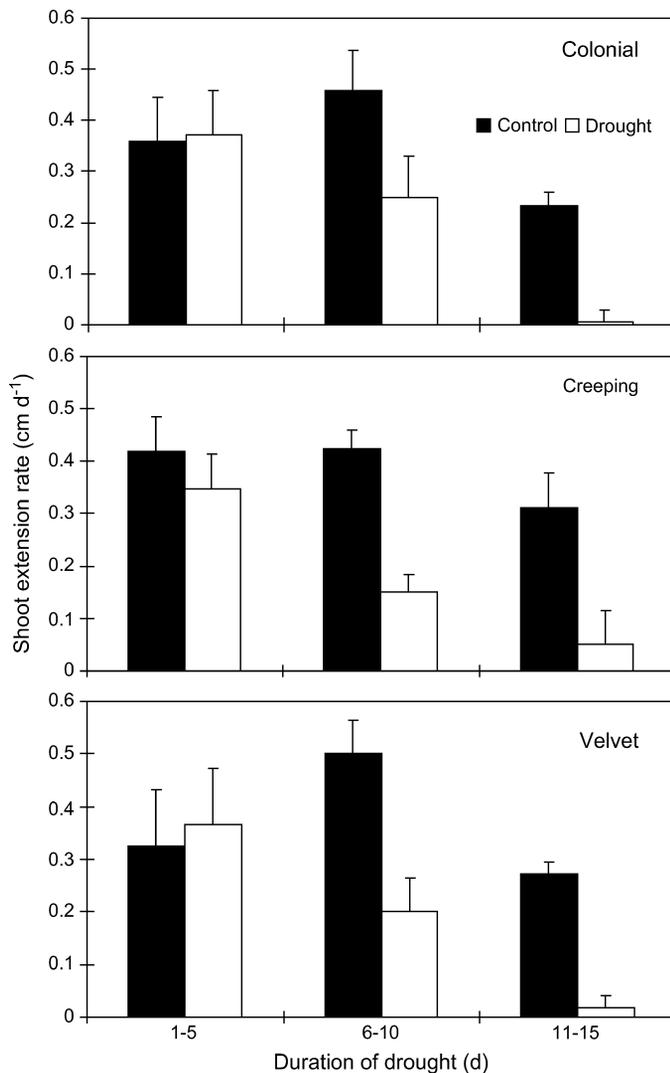
Fig. 3. Shoot extension rates for colonial bentgrass, creeping bentgrass, and velvet bentgrass under drought stress. Data presented are average shoot extension rates for 1 to 5, 6 to 10, and 11 to 15 d of drought treatment. Treatment means for each species are the average of five measurements (replicates). Vertical bars are LSD values ($P \leq 0.05$) indicating statistically significant differences for treatment comparisons for a given 5-d period.

significantly higher ET rates at 10, 13, and 16 d of drought treatment compared with the other two species (Fig. 5).

In general, ET recovered more rapidly than Pn, with ET increasing to ≈87%, 78%, and 94% of prestress levels for colonial bentgrass, creeping bentgrass, and velvet bentgrass respectively by 5 d of rewatering (Fig. 5). At this duration of the recovery period, Pn did not recover for any of the species. In addition, velvet bentgrass exhibited more rapid recovery of ET compared with colonial bentgrass and creeping bentgrass as demonstrated at 5 and 10 d of the recovery treatment period. Canopy net photosynthetic rate recovered to almost 100% of prestress levels by 10 d of rewatering for colonial bentgrass, and at 17 d of rewatering for creeping bentgrass. Although velvet bentgrass did not fully recover to 100% of prestress levels, there were no statistical differences in Pn recovery among the three species at either 10 or 17 d of rewatering.

Under well-watered conditions, there were no differences in ABA accumulation among the bentgrass species, which was
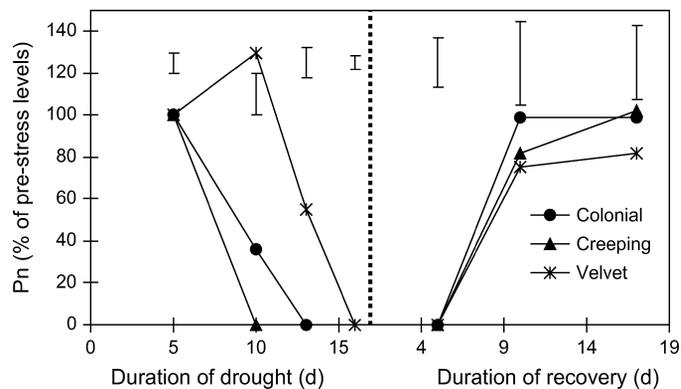

Fig. 4. Canopy photosynthesis, expressed as a percentage of prestress levels, for colonial bentgrass, creeping bentgrass, and velvet bentgrass during drought and recovery treatments. The dashed line separates the drought and recovery periods. Treatment means for each species are the average of five measurements (replicates). Vertical bars are LSD values ($P \leq 0.05$) indicating statistically significant differences for species comparisons at a given day of treatment.

maintained at ≈34 pmol·g^{-1} dry weight throughout the treatment period (data not shown). Abscisic acid content increased from ≈34 pmol·g^{-1} dry weight at 1 d of treatment to 100 pmol·g^{-1} dry weight at 9 d of treatment for all three bentgrass species (Fig. 6). During this time, there were no significant differences in ABA accumulation among the species. By 14 d of drought treatment, ABA content increased to 185 and 223 pmol·g^{-1} dry weight for colonial bentgrass and creeping bentgrass, which was significantly higher than the ABA content for velvet bentgrass (127 pmol·g^{-1} dry weight). Upon 12 d of rewatering, ABA content was significantly lower for colonial bentgrass compared with both creeping bentgrass and velvet bentgrass at 12 d of rewatering. By 16 d of rewatering, ABA content declined to prestress levels, with no significant differences observed among the three species.

Under well-watered conditions, iPA accumulation for all three species was maintained at ≈2 pmol·g^{-1} dry weight throughout the treatment period (data not shown). At 6 d of drought treatment, colonial bentgrass exhibited higher iPA
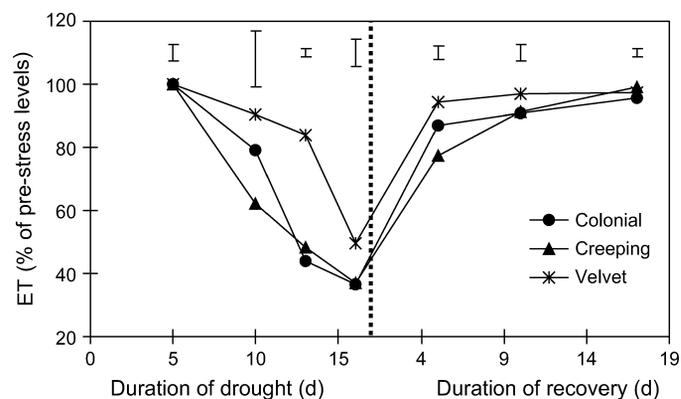

Fig. 5. Canopy ET, expressed as a percentage of prestress levels, for colonial bentgrass, creeping bentgrass, and velvet bentgrass during drought and recovery treatments. The dashed line separates the drought and recovery periods. Treatment means for each species are the average of five measurements (replicates). Vertical bars are LSD values ($P \leq 0.05$) indicating statistically significant differences for species comparisons at a given day of treatment.
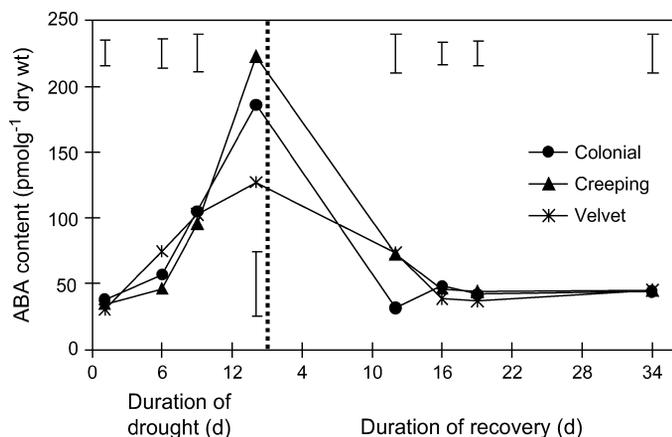
Fig. 6. Abscisic acid content under drought stress and rewatering for colonial bentgrass, creeping bentgrass, and velvet bentgrass. The dashed line separates the drought and recovery periods. Treatment means for each species are the average of five measurements (replicates). Abscisic acid content for well-watered control plants were maintained at ≈34 pmol·g^{-1} dry weight (data not shown), regardless of species. Vertical bars are LSD values ($P \leq 0.05$) indicating statistically significant differences for species comparisons at a given day of treatment.

content (5.3 pmol·g^{-1} dry weight) compared with creeping bentgrass (2.5 pmol·g^{-1} dry weight) and velvet bentgrass (3 0.1 pmol·g^{-1} dry weight; Fig. 7). By 14 d of drought, iPA content declined to between 0 and 0.5 pmol·g^{-1} dry weight, with no significant differences observed among the species. Upon rewatering, iPA content increased to the highest levels for colonial bentgrass (3.7 pmol·g^{-1} dry weight) by 12 d of rewatering compared with creeping bentgrass and velvet bentgrass. By 16 d of rewatering, there were no significant differences among the species, with an average iPA accumulation of 1.6 pmol·g^{-1} dry weight.

Discussion

Drought stress resulted in significant declines in TQ, shoot extension rates, Pn, ET, and cytokinin content, and an increase
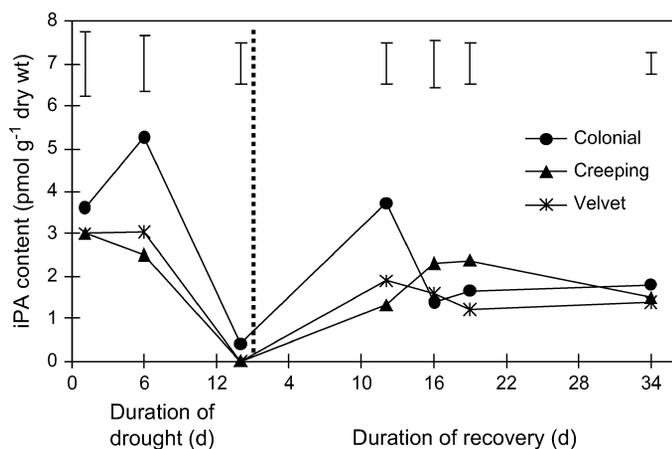

Fig. 7. Isopentyl adenosine content under drought stress and rewatering for colonial bentgrass, creeping bentgrass, and velvet bentgrass. The dashed line separates the drought and recovery periods. Treatment means for each species are the average of five measurements (replicates). Isopentyl adenosine content for well-watered control plants were maintained at ≈2 pmol·g^{-1} dry weight (data not shown), regardless of species. Vertical bars are LSD values ($P \leq 0.05$) indicating statistically significant differences for species comparisons at a given day of treatment.

in ABA content for all three bentgrass species. Velvet bentgrass exhibited less severe or delayed physiological injury resulting from drought stress, as exhibited by prolonged maintenance of higher TQ and gas exchange parameters compared with colonial bentgrass and creeping bentgrass. Upon rewatering after drought stress, colonial bentgrass exhibited a more rapid recovery in turfgrass growth and water use compared with creeping bentgrass and velvet bentgrass. Although recovery in TQ could be associated with increased Pn upon rewatering, there were no significant differences in Pn recovery between the three bentgrass species. Abscisic acid and cytokinin contents, however, varied between species during drought and rewatering, which could be associated with variability in turf performance among the three bentgrass species under drought stress and during recovery after drought.

Increases in ABA content in response to drought stress may reduce stomatal conductance, transpiration, and leaf growth (Blackman and Davies, 1985; Davies et al., 2002; Zhang and Davies, 1989). High stomatal resistance by partial stomatal closure may protect plants from drought injury by water conservation (Zhang and Kirkham, 1995). The extent and rate of ABA accumulation varies with species or cultivars differing in drought tolerance (Conti et al., 1994; Pekic et al., 1995). Previous studies with annual crops have demonstrated a relationship between low ABA accumulation and drought tolerance (Durley et al., 1983; Innes et al., 1984; Landi et al., 2001). A drought-tolerant cultivar of orchardgrass exhibited lower leaf ABA accumulation compared with a drought-susceptible cultivar (Volaire et al., 1998). Wang and Huang (2003) and Wang et al. (2003) also found that cultivars of Kentucky bluegrass exhibiting less ABA accumulation had better TQ, water relations, and photosynthesis under short-term drought stress compared with drought-susceptible cultivars that accumulated greater leaf ABA content. In the current study, ABA content increased with drought stress, which was associated with decreases in ET and shoot extension rate for all three bentgrass species. During the drought treatment, however, velvet bentgrass had significantly less ABA accumulation compared with colonial bentgrass and creeping bentgrass, suggesting less drought injury. Enhanced ABA accumulation for colonial bentgrass and creeping bentgrass could be associated with lower stomatal conductance, and could explain earlier declines in Pn and ET compared with velvet bentgrass. Maintenance of higher levels of Pn and ET for greater duration of drought could support the continued assimilation of carbon and sustained plant survival during drought stress.

Cytokinins have antagonistic effects to ABA, including increasing transpiration rates, reversing ABA-induced stomatal closure and delaying leaf senescence (Blackman and Davies, 1983; Naqvi, 1999; Pospíšilová et al., 2000). During drought stress, delivery of cytokinins typically declines from roots to shoots (Shashidhar et al., 1996). Bano et al. (1993) demonstrated significant decreases in both concentration and delivery of two cytokinins (ZR and iPA) in drought-stressed rice (*Oryza sativa* L.) plants. Our results also support these previous findings, with iPA content decreasing by more than 80% under prolonged drought treatment for all three bentgrass species. Declines in cytokinin content led to increased ABA-to-cytokinin ratios, which in turn can amplify shoot responses to increased levels of ABA (Davies and Zhang, 1991). The application of ABA alone to sunflower (*Helianthus annuus* L.) plants caused a decline in transpiration; however, when ABA and ZR

were applied together, the effects of ABA on transpiration were significantly reduced, even when ZR concentrations were 100 times less than that of ABA (Hansen and Dorffing, 2003). This suggests that even small changes in cytokinin concentration or delivery to leaves can have drastic effects on the physiological responses of plants to environmental stresses.

When plants were rewatered after drought stress, ABA levels dropped and iPA content increased to prestress levels. As discussed previously, ABA is considered a growth inhibitor whereas cytokinins act as growth promoters. Therefore, the decline in ABA content in combination with enhanced cytokinin content in leaves of previously stressed plants could contribute to the recovery of leaf growth, photosynthesis, stomatal conductance, and transpiration rates, which is similar to what has been observed in other plant species (Blackman and Davies, 1985; Hansen and Dorffing, 2003; Ivanova et al., 1998; Yordanov et al., 1997). In general, ABA decreased and iPA levels increased more rapidly and to a greater extent for colonial bentgrass compared with both creeping bentgrass and velvet bentgrass when rewatered, which could have contributed to better recovery of colonial bentgrass compared with creeping bentgrass and velvet bentgrass.

In summary, lower ABA accumulation under drought stress was associated with higher drought adaptability for velvet bentgrass, as exhibited by prolonged TQ and gas exchange parameters under drought stress compared with colonial bentgrass and creeping bentgrass. A rapid decline in ABA content and an increase in cytokinin content upon rewatering was important for recovery of bentgrasses from drought stress. Colonial bentgrass exhibited more rapid changes in hormone balance to prestress levels, which was associated with improved quality characteristics and resumption of gas exchange parameters after drought stress.

Literature Cited

Alves, A.A.C. and T.L. Setter. 2000. Response of cassava to water deficit: Leaf area growth and abscisic acid. Crop Sci. 40:131–137.

Assmann, S.M. and K.L. Shimazaki. 1999. The multisensory guard cell, stomatal responses to blue light and abscisic acid. Plant Physiol. 119:809–816.

Bacon, M.A., S. Wilkinson, and W.J. Davies. 1998. pH-Regulated leaf cell expansion in droughted plants is abscisic acid dependent. Plant Physiol. 118:1507–1515.

Bano, A., K. Dorffling, D. Bettin, and H. Hahn. 1993. Abscisic acid and cytokinins as possible root-to-shoot signals in xylem sap of rice plants in drying soil. Aust. J. Plant Physiol. 20:109–115.

Barrs, H.D. and P.E. Weatherley. 1962. A re-examination of the relative turgidity technique for estimating water deficit in leaves. Aust. J. Biol. Sci. 15:413–428.

Beck, E. 1996. Regulation of shoot/root ratio by cytokinins from roots of *Urtica dioica*: Opinion. Plant Soil 185:3–12.

Bellaire, B.A., J. Carmody, J. Braud, D.R. Gosset, S.W. Banks, M.C. Lucas, and T.E. Fowler. 2000. Involvement of abscisic acid-dependent and -independent pathways in the up-regulation of antioxidant enzyme activity during NaCl stress in cotton callus tissue. Free Radic. Res. 33:531–545.

Blackman, P.G. and W.J. Davies. 1983. The effects of cytokinins and ABA on stomatal behavior of maize and *Commelina*. J. Expt. Bot. 34:1619–1626.

Blackman, P.G. and W.J. Davies. 1985. Root to shoot communication in maize plants of the effects of soil drying. J. Expt. Bot. 36:39–48.

Bray, E.A., T.Y. Shih, M.S. Moses, A. Cohen, R. Imai, and A.L. Plant. 1999. Water deficit induction of a tomato H1 histone requires abscisic acid. Plant Growth Regul. 29:35–46.

Bueno, P., A. Piqueras, J. Kurepa, A. Savoure, N. Verbruggen, M. VanMontegu, and D. Inze. 1998. Expression of antioxidant enzymes in response to abscisic acid and high osmoticum in tobacco BY-2 cell cultures. Plant Sci. 138:27–34.

Campbell, J.L., N.Y. Klueva, K. Zheng, J. Nieto–Sotelo, T.H.D. Ho, and H.T. Nguyen. 2001. Cloning of new members of heat shock protein HSP101 gene family in wheat (*Triticum aestivum* (L.) Moench) inducible by heat, dehydration, and ABA. Biochim. Biophys. Acta 1517:270–277.

Čatský, J., J. Pospíšilová, M. Kamíinek, A. Gaudinová, J. Pulkrábek, and J. Zahradníček. 1996. Seasonal changes in sugar beet photosynthesis as affected by exogenous cytokinin N6-(*m*-hydroxybenzyl)-adenosine. Biol. Plant. 38:511–518.

Cheikh, N. and R.J. Jones. 1994. Disruption of maize kernel growth and development by heat stress. Role of cytokinin/abscisic acid balance. Plant Physiol. 106:45–51.

Conti, S., P. Landi, M.C. Sanguineti, S. Stefanelli, and R. Tuberosa. 1994. Genetic and environmental effects on abscisic acid accumulation in leaves of field-grown maize. Euphytica 78:81–89.

Davies, W.J. and J. Zhang. 1991. Root signals and the regulation of growth and development of plants in drying soil. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:55–76.

Davies, W.J., S. Wilkinson, and B. Loveys. 2002. Stomatal control by chemical signaling and the exploitation of this mechanism to increase water use efficiency in agriculture. New Phytol. 153:449–460.

Durley, R.C., T. Kannangara, N. Seetharama, and G.M. Simpson. 1983. Drought resistance of sorghum bicolor. 5. Genotypic differences in the concentrations of free and conjugated abscisic, phaseic, and indole-3-acetic acids in leaves of field-grown drought-stressed plants. Can. J. Plant Sci. 63:131–145.

Goicoechea, N., M.C. Antolin, and M. Sanchez–Diaz. 1997. Gas exchange is related to the hormonal balance in mycorrhizal or nitrogen-fixing alfalfa subjected to drought. Physiol. Plant. 100:989–997.

Goicoechea, N., K. Dolezal, M.C. Antolin, M. Strnad, and M. Sanchez–Diaz. 1995. Influence of mycorrhizae and *Rhizobium* on cytokinin content in drought-stressed alfalfa. J. Expt. Bot. 46:1543–1549.

Hansen, H. and K. Dorffing. 2003. Root-derived *trans*-zeatin riboside and abscisic acid in drought-stressed and rewatered sunflower plants: Interaction in the control of leaf diffusive resistance? Funct. Plant Biol. 30:365–375.

Henson, I.E. and N.C. Turner. 1991. Stomatal responses to abscisic acid in three lupin species. New Phytol. 117:529–534.

Henson, I.E., V. Mahalakshmi, F.R. Bidinger, and G. Alagarswamy. 1981. Genotypic variation in pearl millet (*Pennisetum americanum* (L.) Leeke), in the ability to accumulate abscisic acid in response to water. J. Expt. Bot. 32:899–910.

Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil, p. 31. Calif. Agr. Expt. Sta. Circ. 347. College of Agriculture, University of California, Berkeley, Calif.

Ilahi, I. and K. Dorffling. 1982. Changes in abscisic acid and proline levels in maize varieties of different drought resistance. Physiol. Plant 55:129–135.

Incoll, L.D. and P.C. Jewer. 1987. Cytokinins and stomata, p. 281–292. In: E. Zeiger, G.D. Farquhar, and I.R. Cowan (eds.). Stomatal function. Stanford University Press, Palo Alto, Calif.

Innes, P., R.D. Blackwell, and S.A. Quarrie. 1984. Some effects of genetic variation in drought-induced abscisic acid accumulation on the yield and water use of spring wheat. J. Agr. Sci. 102:341–351.

Ivanova, A.P., K.L. Stefanov, and I.T. Yordanov. 1998. Effect of cytokinin 4-PU-30 on the lipid composition of water stressed soybeans. Biol. Plant. 41:155–159.

Jewer, P.C. and L.D. Incoll. 1980. Promotion of stomatal opening in the grass *Anthephora pubescens* Nees by a range of natural and synthetic cytokinins. Planta 150:218–221.

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Jiang, M. and J. Zhang. 2002. Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. J. Expt. Bot. 53:2401–2410.

Jin, S., C.C.S. Chen, and A.L. Plant. 2000. Regulation by ABA of osmotic-stress-induced changes in protein synthesis in tomato roots. Plant Cell Environ. 23:51–60.

Kneebone, W.R., D.M. Kopec, and C.F. Mancino. 1992. Water requirement and irrigation, p. 441–472. In: D.V. Waddington, R.N. Carrow, and R.C. Shearman (eds.). Turfgrass agronomy monograph no. 32. ASA, CSSA, SSSA, Madison, Wisc.

Landi, P., M.C. Sanguineti, S. Conti, and R. Tuberosa. 2001. Direct and correlated responses to divergent selection for leaf abscisic acid concentration in two maize populations. Crop Sci. 41:335–344.

Larque–Saavedra, A. and R.L. Wain. 1976. Studies on plant growth-regulating substances. XLII. Abscisic acid as a genetic character related to drought tolerance. Ann. Appl. Biol. 83:291–297.

Lechowski, Z. 1997. Stomatal response to exogenous cytokinin treatment of the hemiparasite *Melampyrum arvense* L. before and after attachment to the host. Biol. Plant. 39:13–21.

Liu, X. and B. Huang. 2005. Root physiological factors involved in cool-season grass response to high soil temperature. Environ. Exp. Bot. 53:233–245.

Liu, X., B. Huang, and G. Banowetz. 2002. Cytokinin effects on creeping bentgrass responses to heat stress: I. Shoot and root growth. Crop Sci. 42:457–465.

Moore-Gordon, C.S., A.K. Cowan, I. Bertling, C.E.J. Botha, and R.H.M. Cross. 1998. Symplastic solute transport and avocado fruit development: A decline in cytokinin/ABA ratio is related to appearance of the Hass small fruit variant. Plant Cell Physiol. 39:1027–1038.

Naqvi, S.S.M. 1995. Plant/crop hormones under stressful conditions, p. 645–660. In: M. Pessarakli (ed.). Handbook of plant and crop stress. Marcel Dekker, New York.

Naqvi, S.S.M. 1999. Plant hormones and stress phenomena, p. 709–730. In: M. Pessarakli (ed.). Handbook of plant and crop stress. Marcel Dekker, New York.

Pekic, S., R. Stikic, L. Tomljanovic, V. Andjelkovic, M. Ivanovic, and S.A. Quarrie. 1995. Characterization of maize lines differing in leaf abscisic acid content in the field. 1. Abscisic acid physiology. Ann. Bot. (Lond.) 75:67–73.

Pospíšilová, J., H. Synková, and J. Rulcová. 2000. Cytokinins and water stress. Biol. Plant. 43:321–328.

Quarrie, S.A. 1989. Abscisic acid as a factor in modifying drought resistance, p. 27–37. In: J.H. Cherry (ed.). Environmental stress in plants: Biochemical and physiological mechanisms. Springer-Verlag, Berlin.

Quarrie, S.A. 1993. Understanding plant responses to stress and breeding for improved stress resistance: The generation gap, p. 224–245. In: T.J. Close and E.A. Bray (eds.). Plant responses to cellular dehydration during environmental stress. Proc. 16th Annu. Riverside Symp. Plant Physiol., vol. 10. Riverside, Calif.

Setter, T.L., B.A. Flannigan, and J. Melkonian. 2001. Loss of kernel set due to water deficit and shade in maize: Carbohydrate supplies, abscisic acid, and cytokinins. Crop Sci. 41:1530–1540.

Shashidhar, V.R., T.G. Prasad, and L. Sudharshan. 1996. Hormone signals from roots to shoots of sunflower (*Helianthus annuus* L.). Moderate soil drying increases delivery of abscisic acid and depresses delivery of cytokinins in xylem sap. Ann. Bot. (Lond.) 78:151–155.

Synková, H., N. Wilhelmová, Z. Šesták, and J. Pospíšilová. 1997. Photosynthesis in transgenic plants with elevated cytokinin contents, p. 541–552. In: M. Pessarakli (ed.). Handbook of photosynthesis. Marcel Dekker, New York.

Tamminen, I., P. Makela, P. Heino, and E.T. Palva. 2001. Ectopic expression of ABI3 gene enhances freezing tolerance in response to abscisic acid and low temperature in *Arabidopsis thaliana*. Plant J. 25:1–8.

Topp, G.C., J.L. Davis, and A.P. Annan. 1980. Electromagnetic determination of soil water content: Measurements in coaxial transmission lines. Water Resour. Res. 16:574–582.

Volaire, F., H. Thomas, N. Bertagne, E. Bourgeois, M.F. Gautier, and F. Lelievre. 1998. Survival and recovery of perennial forage grasses under prolonged Mediterranean drought: Water status, solute accumulation, abscisic acid concentration and accumulation of dehydrin transcripts in bases of immature leaves. New Phytol. 140: 451–460.

Wang, Z. and B. Huang. 2003. Genotypic variation in abscisic acid accumulation, water relations, and gas exchange for Kentucky bluegrass exposed to drought stress. J. Amer. Soc. Hort. Sci. 128: 349–355.

Wang, Z., B. Huang, and Q. Xu. 2003. Effects of abscisic acid on drought responses of Kentucky bluegrass. J. Amer. Soc. Hort. Sci. 128:36–41.

Wang, Z., Q. Xu, and B. Huang. 2004. Endogenous cytokinin levels and growth responses to extended photoperiods for creeping bentgrass under heat stress. Crop Sci. 44:209–213.

Wilkinson, S. and W.J. Davies. 2002. ABA-based chemical signaling: The coordination of responses to stress in plants. Plant Cell Environ. 25:195–210.

Yordanov, I., T. Tsonev, V. Goltsev, M. Merakchiiska-Nikolova, and K. Georgieva. 1997. Gas exchange and chlorophyll fluorescence during water and high temperature stresses and recovery. Probable protective effect of carbamide cytokinin 4-PU30. Photosynthetica 33:423–431.

Yordanov, I., V. Velikova, and T. Tsonev. 1999. Influence of drought, high temperature, and carbamide cytokinin 4-PU-30 on photosynthetic activity of bean plants. 1. Changes in chlorophyll fluorescence quenching. Photosynthetica 37:447–457.

Zhang, J. and W.J. Davies. 1989. Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. Plant Cell Environ. 12:73–81.

Zhang, X. and E.H. Ervin. 2004. Cytokinin-containing seaweed and humic acid extracts associated with creeping bentgrass leaf cytokinins and drought resistance. Crop Sci. 44:1737–1745.

Zhang, J. and M.B. Kirkham. 1995. Water relations, water-stressed split-root C4 (*Sorghum bicolor*, Poaceae) and C3 (*Helianthus annuus*, Asterceae) plants. Amer. J. Bot. 82:1220–1229.