Genotypic Variation in Abscisic Acid Accumulation, Water Relations, and Gas Exchange for Kentucky Bluegrass Exposed to Drought Stress

ZhaoLONG Wang1 and Bingru Huang2
Department of Plant Biology and Pathology, Rutgers University, New Brunswick, NJ 08901.

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ABSTRACT. Drought is a major limiting factor for turfgrass growth. Understanding genetic variations in physiological responses of turfgrass to drought stress would facilitate breeding and management programs to improve drought resistance. This study was designed to evaluate responses of abscisic acid (ABA) accumulation, water relations, and gas exchange to drought stress in four Kentucky bluegrass (Poa pratensis L.) cultivars differing in drought resistance. Plants of 'Midnight' and 'A82-204' (drought resistant) and 'Brilliant' and 'RSP' (drought susceptible) were grown under well-watered (control) or drought stress conditions for 25 days in growth chambers. Turf quality, leaf water potential (ψleaf), relative water content (RWC), leaf net photosynthesis rate (Pn), and stomatal conductance (gs) declined, while electrolyte leakage (EL) increased during drought progression in all cultivars. The magnitudes of the change in these parameters were greater for 'RSP' and 'Brilliant' than for 'Midnight' and 'A82-204'. Leaf ABA content in 'RSP' and 'Brilliant' increased sharply after 2 days of stress to as much as 34 times the control level at 10 days of drought. Leaf ABA content in 'Midnight' and 'A82-204' also increased with drought, but to a lesser extent than in the other two cultivars. Leaf ABA level was negatively correlated with ψleaf and gs. 'A82-204' had a significantly lower ABA accumulation rate with changes in ψleaf during drought compared to 'Midnight', 'RSP' and 'Brilliant'; however, no differences in ABA accumulation rate were detected among the latter three cultivars. In addition, leaf gs was more sensitive to changes in ABA accumulation in 'Midnight' and 'A82-204' than in 'RSP' and 'Brilliant'. These results demonstrated that drought tolerant cultivars of Kentucky bluegrass are characterized by lower ABA accumulation and less severe decline in ψleaf, Ps, gs, and turf quality during drought stress than drought sensitive cultivars. Drought tolerance of Kentucky bluegrass could be related to sensitivity of stomata to endogenous accumulation of ABA under drought stress conditions.

Drought stress can severely limit growth of turfgrasses (Arison et al., 1987; Beard, 1973, 1989), particularly in urban areas where water availability for landscape irrigation is increasingly limited. Drought injury of turfgrasses involves many physiological and biochemical changes (Carrow, 1996; Howard and Watschke, 1991; Huang et al., 1998; Huang and Gao, 1999; Jiang and Huang, 2000, 2001; Perdomo et al., 1996). Stomatal closure is among the earliest plant responses to drought stress, which can subsequently lead to a reduction in photosynthetic rate and water loss (Blum and Simmena, 1995; Raschke and Hedrich, 1985).

Abscisic acid (ABA) is an essential constituent of higher plants. This hormone induces rapid stomatal closure and reduction in plant transpiration, thus lessening the decrease in leaf water potential and protecting leaf tissue from turger loss during drought stress (Borel et al., 1997; Cock et al., 1985; El-Sharkawy and Cock, 1984; Palta, 1984; Trejo et al., 1995; Wang et al., 2002). ABA also induces the expression of several drought-resistant genes by reprogramming the cell to withstand dehydration stress (Bray et al., 1999; Campbell et al., 2001; Furumoto et al., 2000; Jin et al., 2000; Lorenzo et al., 2001; Neill and Burnett, 1999; Tamminen et al., 2001). Genotypic difference in drought tolerance is correlated with changes in ABA concentration and sensitivity in several annual crops (Blum and Simmena, 1995; Cellier et al., 1998). Drought-susceptible varieties have been found to produce higher levels of ABA than drought-resistant varieties in maize (Zea mays L.) (Ilahi and Dorfling, 1982), spring wheat (Triticum aestivum L. Em. Thell.) (Quarrie and Jones, 1979; Quarrie, 1980; 1981), and sorghum (Sorghum bicolor L. Moench.) (Durely et al., 1983). In contrast, accumulation of leaf ABA has been claimed to be positively associated with drought resistance in soybean (Glycine max L. Merr.) (Samet et al., 1980) and millet (Pennisetum americanum L.) (Henson et al., 1981). In spite of the contrary results in different species, leaf ABA accumulation has been used as a selection criterion to improve drought tolerance in the breeding program for cereal crops (Innes et al., 1984; Landi et al., 2001; Quarrie, 1989). The research in ABA associated with drought resistance in annual crops has stimulated our search for genetic variation in ABA accumulation and sensitivity as a means of improving drought resistance in perennial turfgrasses. Such research would help to identify the role of ABA in regulating drought tolerance of turfgrasses and provide guidelines for applications of plant growth regulators in management practices to improve drought resistance.

The objectives of the study were to examine genotypic variation in ABA accumulation and physiological responses to drought stress in four cultivars of Kentucky bluegrass, and to determine the relationship of ABA accumulation and drought resistance.

Materials and Methods

PLANT MATERIALS AND GROWTH CONDITIONS. Two relatively drought-tolerant cultivars (Midnight and A82-204) and two drought-susceptible cultivars (RSP and Brilliant) in Kentucky bluegrass were examined in this study. Sods of equivalent quality (15 cm in diameter and 2 cm thick) were collected from field plots at Adelphia Turfgrass Experiment Station, Rutgers University, N.J. Sods were washed free of soil before planting in plastic pots (20 cm in diameter and 40 cm deep) filled with a mixture of sand and topsoil (fine, montmorillonitic, mesic, aquic argidolls) (1:2:1, v/v). Plants were grown in a greenhouse for 90 d and then transferred to growth chambers with a temperature of 22/18 °C (day/night), 14-h photoperiod, and photosynthetically active radiation (PAR)
of 600 mmol·m⁻²·s⁻¹. Grasses were maintained in growth chambers for 15 d before drought treatment was imposed. Controlled-release fertilizer (17N-6P-10K) was top-dressed twice prior to dry-down to provide a total N of 17 g·m⁻². Turf was hand-clipped twice weekly to a 6-cm height.

**TREATMENTS AND MEASUREMENTS.** The experiment consisted of two soil moisture treatments: a) drought stress (irrigation withheld and soil allowed to dry for 25 d and b) well-watered control (plants irrigated every other day until drainage occurred from the bottom of the pots). Volumetric soil water content in the 0-20 cm soil layer was monitored to determine soil dryness during drought stress by time domain reflectometry (Soil Moisture Equipment Corp., Santa Barbara, Calif.). The field capacity of the sand and topsoil mix was 27% ± 0.5% (mean of eight replications ± standard error), which was measured in eight pots when drainage ceased following watering the soil to complete saturation.

Several physiological measurements were made at 4-d intervals during the experiment. All measurements were made on six young, fully expanded leaves from different plants in each pot. Leaf net photosynthetic rate (Pn) and stomatal conductance (gs) were measured with a portable gas-exchange system (LI-6400; LI-COR Inc., Lincoln, Nebr.) at a PAR of 800 mmol·m⁻²·s⁻¹ and a chamber temperature of 22 °C from 1000 to 1400 HR at each measurement time. Turf quality was rated visually based on color, density, and uniformity on a scale of 1 (worst, most plants died) to 9 (best, healthy and green plants). Grasses rated at 6 or above were considered to have acceptable quality. Leaf relative water content (RWC) was calculated based on leaf fresh weight, dry weight, and turgid weight measured after soaking leaves in water for 8 h. Leaf samples were dried at 80°C in an oven for 72 h and then dry weight was determined. Six young, fully expanded leaves from different plants in each pot were collected for water potential measurement. Water potential (Ψleaf) of individual leaves was measured using a thermocouple psychrometer (Tru Psi, Decagon Devices, Inc., Pullman, Wash.). Cell membrane stability was estimated by measuring electrolyte leakage (EL). Samples of 0.1 g leaves were rinsed and immersed in 20 mL of deionized water. The conductivity of the solution (Cinit) was measured after the leaves were shaken for 24 h. Leaves then were killed by autoclaving at 140 °C for 20 min. The conductivity of killed tissues (Cmax) was measured after samples were cooled to the room temperature. Relative EL was calculated as (Cinit/Cmax) × 100.

**ABSCISIC ACID EXTRACTION AND ANALYSIS.** Extraction and analysis of abscisic acid followed the methods described by Alves and Setter (2000). Fully expanded leaves were extracted in 80% [v/v] methanol (with 1% glacial acetic acid [v/v] and 10 mg·L⁻¹ butylated hydroxytoluene, sample:extraction = 1:10 [w/v]). After centrifuged at 10,000 g for 10 min, the supernatants were vacuum-dried and resuspended in 20% methanol. Aliquots (100 mL) were applied onto C18 chromatography columns (25 mg, Supelco, Bellefonte, Pa.) for purification. Hydrophilic contaminants were washed out with 200 mL 20% methanol. The ABA-containing fraction was eluted with 200 mL 50% methanol (with 1% glacial acetic acid, v/v), vacuum-dried, and redissolved in Tris-buffered saline (TBS) solution (pH 7.5). Thirty Bq of 3H-ABA (A 7090, Sigma Co., Mo.) was added to each sample to monitor loss of ABA during the purification. ABA was quantified by using anti-ABA monoclonal antibody (Agdia Inc., Elkhart, Ind.) by indirect enzyme linked immunosorbant assay (ELISA) as previously described by Alves and Setter (2000).

**EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS.** The experiment consisted of two factors (four cultivars and two soil moisture treatments) with four replications arranged in a completely randomized design. Treatment effects were determined by analysis of variance according to the general linear model procedure of the Statistical Analysis System (SAS Institute, Cary, N.C.). Differences among cultivar means were separated by the least significant difference (LSD, P ≤ 0.05) test and the LSD values are illustrated in the figures. The relationships between leaf water potential and ABA content and between stomatal conductance and ABA content were determined using the linear regression analysis and the significance of the correlation coefficients and the differences in the slopes of the relationships were tested at a probability level of 0.05.

**Results**

**LEAF ABA ACCUMULATION.** Under well-watered conditions, leaf ABA content remained a low level in all cultivars, and no cultivar differences were observed (Fig. 1A). Leaf ABA content increased with drought duration (Fig. 1B). There was a 13-fold increase in ABA content in ‘RSP’ and ‘Brilliant’ after 4 d of drought and up to a 34-fold increase after 12 d. ABA contents in ‘Midnight’ and ‘A82-204’ did not show significant increases until 4 d. ‘RSP’ and ‘Brilliant’ had significantly higher ABA contents than ‘Midnight’ and ‘A82-204’, beginning at 4 d of drought, and their ABA content was about three times greater than that of ‘Midnight’ and ‘A82-204’ by 10 d of drought.

**WATER STATUS AND RELATION TO ABA ACCUMULATION.** Soil water content declined rapidly from 27% to 11% within the first...
8 d of drought stress and to ≈5% after 12 d and remained at this level until the end of the experiment (24 d) (Fig. 2). Soil drying rates were similar for all four cultivars.

Leaf water potential (ψₑₑₑₑ) and RWC remained constant during the experimental period, and cultivars were not different in either parameter under well-watered conditions (Fig. 3A and C). ψₑₑₑₑ and RWC declined with progressive drought in all cultivars, and the decline was more pronounced for ‘RSP’ and ‘Brilliant’ than for ‘Midnight’ and ‘A82-204’ (Fig. 3B and D). ‘Midnight’ and ‘A82-204’ maintained higher ψₑₑₑₑ and RWCs than ‘RSP’ and ‘Brilliant’, beginning 4 d after drought stress. By the end of the experiment, almost all leaves of ‘RSP’ and ‘Brilliant’ were desiccated and brown, while some leaves of ‘Midnight’ and ‘A82-204’ remained turgid and green.

ABA content in leaves of stressed plants was negatively and linearly correlated with ψₑₑₑₑ for all cultivars (Fig. 4). No significant differences were detected in the slopes of the lines for ‘Midnight’, ‘RSP’ and ‘Brilliant’. The slope of the line for ‘A82-204’ was significantly less steep than those for the other three cultivars.

Photosynthesis and Stomatal Conductance in Relation to ABA Accumulation. Leaf Pn remained constant for all cultivars under well-watered conditions, and ‘Midnight’ had the lowest Pn among the four cultivars (Fig. 5A). Pn declined to below the initial level after 8 d of drought stress for all cultivars. The decline in Pn for ‘RSP’ and ‘Brilliant’ was 80% while the decline in Pn for ‘Midnight’ and ‘A82-204’ was 50% at 8 d of stress. ‘RSP’ and ‘Brilliant’ had significantly lower Pn than ‘Midnight’ at 8, 12, and 16 d of stress and ‘A82-204’ at 8 and 16 d, but not after 12 d of stress (Fig. 5B).

Under well-watered conditions, ‘A82-204’ had higher gₑ than the other three cultivars. No significant difference in gₑ was observed among ‘Midnight’, ‘RSP’, and ‘Brilliant’ (Fig. 6A). Stomatal conductance declined significantly at 4 d of drought stress, compared to the initial level and reached the lowest level at 8 d for ‘Midnight’ and ‘A82-204’ and 12 d for ‘RSP’ and ‘Brilliant’. ‘RSP’ and ‘Brilliant’ had higher gₑ than ‘Midnight’ and ‘A82-204’ at 8 d, but lower gₑ at 12 d of drought (Fig. 6B).

Stomatal conductance was negatively and line-early correlated with ABA content in leaves of stressed plants for all cultivars during the first 12 d of drought stress (Fig. 7). The slopes of the lines differed for the drought-sensitive and drought-tolerant cultivars. The slopes of the lines for ‘Midnight’ and ‘A82-204’ were significantly greater than those for ‘RSP’ and ‘Brilliant’.

Turf Quality and Membrane Stability. The initial level of turf quality was approximately 8.0 for all four cultivars under well-watered conditions (Fig. 8A). When plants were subjected to drought stress, turf quality declined for all cultivars, but to a greater extent for ‘RSP’ and ‘Brilliant’ than for ‘Midnight’ and ‘A82-204’ (Fig. 8B). ‘Midnight’ remained at the acceptable level (turf quality = 6) during most of the experimental period, while turf quality of ‘RSP’ and ‘Brilliant’ declined to below the acceptable level at 12 d of stress. ‘Midnight’ and ‘A82-204’ had significantly higher turf quality than ‘RSP’ and ‘Brilliant’, beginning at 8 d of drought.

Leaf EL increased with drought stress in all cultivars (Fig. 9A). The increase in EL for ‘RSP’ and ‘Brilliant’ was more pronounced than that for ‘Midnight’ and ‘A82-204’. Leaf EL for ‘Midnight’ and ‘A82-204’ did not increase above the initial level until 16 d of drought (Fig. 9B). ‘RSP’ and ‘Brilliant’ had significantly higher EL than ‘Midnight’ and ‘A82-204’, beginning of 4 d of drought.

Discussion

Abscisic acid increased significantly with drought in all four cultivars of Kentucky bluegrass studied. Although such increases in ABA have been frequently documented in other species (Alves and Setter, 2000; Cao et al., 2000; Jin et al., 2000; Li et al., 2000; Ober et al., 1991; Sharp et al., 1994; Stoll et al., 2000), few studies have documented changes in ABA levels with drought in turfgrasses. Kentucky bluegrass cultivars differing in physiological responses to drought showed different patterns of ABA accumulation in leaves during drought stress. Leaf ABA content
increased more dramatically in ‘RSP’ and ‘Brilliant’ which showed more rapid and severe decline in turf quality, \( \psi_{\text{leaf}} \), RWC, \( Pn \), \( gs \) and cell membrane stability than that in ‘Midnight’ and ‘A82-204’. The large variation among genotypes in the rate of drying prevented measurements of ABA content for each genotype at the same level of internal water stress. Stikic and Davies (2000) reported more rapid accumulation of ABA in drought susceptible than resistant lines of maize (\( Zea mays \)).

Stomatal conductance of drought resistant cultivars (Midnight and A82-204) declined to a level lower than that of the two sensitive cultivars (Brilliant and RSP) at 8 d of drought when soil moisture was \( \approx 12\% \), but was maintained at a higher level during the remaining stress period (12 to 16 d) when soil moisture decreased to 6%. Stomatal conductance of ‘Brilliant’ and ‘RSP’ rapidly dropped to near zero by 12 d. Complete stomatal closure for ‘RSP’ and ‘Brilliant’ at 12 and 16 d could have resulted from leaf desiccation, which was indicated by their extremely low water content (below 30%) and water potential (below \(-4 \) MPa) at this time of drought stress (Fig. 3). The rapid decline in stomatal conductance or stomata closure at the earlier time, or mild drought stress, for ‘Midnight’ and ‘A82-204’ may lead to less transpiration, reducing drought damage during the prolonged stress period. This was supported by the data on leaf water status and EL. EL of ‘Brilliant’ and ‘RSP’ increased rapidly at 12 and 16 d, while EL of ‘Midnight’ and ‘A82-204’ still remained at a relatively lower level (Fig. 8). The maintenance of stomatal conductance for ‘Midnight’ and ‘A82-204’ during prolonged, severe drought stress would help plants to maintain photosynthesis, and thus to survive prolonged drought. In fact, net photosynthetic rates of ‘Midnight’ and ‘A82-204’ were 4.2 and 0.8 mmol CO\(_2\)/m\(^2\)/s, respectively, at 16 d of drought, while \( Pn \) became negative for both ‘RSP’ and ‘Brilliant’ which \( gs \) dropped to zero during prolonged drought.

ABA accumulation has been found to protect plants from damage by drought by inducing stomatal closure, thus reducing water loss via transpiration during early or mild drought stress (Harris and Outlaw, 1991; Wilkinson and Davies, 1997). ABA regulation of drought tolerance may involve both the sensitivity of ABA biosynthesis to changes in plant water status and the sensitivity of the stomata to the changes in ABA concentration (Quarrie and Jones, 1979). The rates of change in \( gs \) with leaf ABA concentration for ‘Midnight’ and ‘A82-204’ were significantly greater than those

**Fig. 4.** The relationship between leaf ABA content and leaf \( \psi_{\text{leaf}} \) in drought-stressed plants of four cultivars. Lines are fitted linear regressions. \( df = 26 \). Correlation coefficients \( (r) \) are indicated. The difference in the slopes of each cultivar was tested by ANOVA \( (P = 0.05) \).

**Fig. 5.** Responses of single leaf photosynthetic rate \( (Pn) \) to drought stress. Vertical bars are LSD values \( (P = 0.05) \) for treatment and cultivar comparisons at a given day of treatment.
for ‘RSP’ and ‘Brilliant’ (Fig. 7), which indicated that stomata in leaves of drought-resistant cultivars were more sensitive to ABA accumulation than in drought-susceptible cultivars of Kentucky bluegrass.

The sensitivity of stomata to ABA changes also varied with genotype in other species (Cellier et al., 1998; Quarrie and Jones, 1979). The high ABA sensitivity of drought-resistant cultivars may lead to early stomatal closure during short-term drought and limit further water loss, thus lessening damage to cell membranes and turf quality decline caused by soil drying. However, effects of prolonged or severe drought on leaf water status and gs may be ABA-independent and controlled by other factors (Holbrook et al., 2002; Ridolfi et al., 1996). Under extended or severe drought conditions, many other factors, such as osmotic adjustment associated with solute accumulation, contribute to the maintenance of cell turgor, gs, and water status in plants (Bohnert and Jensen, 1996; Nilsen and Orcutt, 1996; Zhang and Archbold, 1993a, 1993b).

In conclusion, drought tolerant cultivars of Kentucky bluegrass were characterized by lower ABA accumulation and less severe decline in $\psi_{\text{m}}$, $P_n$, gs, and turf quality during drought stress. Cultivars differing in drought response varied in stomatal sensitivity to changes in ABA level during drought. Drought tolerance of Kentucky bluegrass could be related to sensitivity of stomata to endogenous accumulation of ABA under short-term or mild drought stress conditions.

**Literature Cited**


Fig. 8. Responses of turf quality to drought stress. Turf quality was rated visually on a scale of one (worst) to nine (best) based on color, uniformity, and density. Grasses rated at six or above were considered to have acceptable quality. Vertical bars on the top or bottom are LSD values (P = 0.05) for cultivar comparisons at a given day of treatment. Vertical bars on the right are LSD values (P = 0.05) for comparisons of changes over treatment duration within the cultivar.

Fig. 9. Responses of Electrolyte leakage (EL) to drought stress. Vertical bars on the top or bottom are LSD values (P = 0.05) for cultivar comparisons at a given day of treatment. Vertical bars on the right are LSD values (P = 0.05) for comparisons of changes over treatment duration within the cultivar.