Osmotic Adjustment in Sorghum

I. Mechanisms of Diurnal Osmotic Potential Changes

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ABSTRACT

Osmotic adjustment, defined as a lowering of osmotic potential (Ψ_Π) due to net solute accumulation in response to water stress, has been considered to be a beneficial drought tolerance mechanism in some crop species. The objective of this experiment was to determine the relative contribution of passive *versus* active mechanisms involved in diurnal Ψ_Π changes in sorghum (*Sorghum bicolor* L. Moench) leaf tissue in response to water stress. A single sorghum hybrid (cv ATx623 × RTx430) was grown in the field under variable water supplies. Water potential, Ψ_Π, and relative water content were measured diurnally on expanding and the uppermost fully expanded leaves before flowering and on fully expanded leaves during the grain-filling period. Diurnal changes in total osmotic potential ($\Delta\Psi_\Pi$) in response to water stress was 1.1 megapascals before flowering and 1.4 megapascals during grain filling in comparison with 0.53 megapascal under well-watered conditions. Under water-stressed conditions, passive concentration of solutes associated with dehydration accounted for 50% (0.55 megapascal) of the diurnal $\Delta\Psi_\Pi$ before flowering and 47% (0.66 megapascal) of the change during grain filling. Net solute accumulation accounted for 42% (0.46 megapascal) of the diurnal $\Delta\Psi_\Pi$ before flowering and 45% (0.63 megapascal) of the change during grain filling in water-stressed leaves. The relative contribution of changes in nonosmotic volume (decreased turgid weight/dry weight) to diurnal $\Delta\Psi_\Pi$ was less than 8% at either growth stages. Water stress did not affect leaf tissue elasticity or partitioning of water between the symplasm and apoplasm.

Loss of water from turgid leaf tissue in response to transpiration results in not only a significant decline in Ψ_w[1] but also a decline in Ψ_Π, to a lesser extent. As the leaf tissue Ψ_w declines even more in response to soil water deficits, Ψ_Π declines accordingly as reported in a large number of crop species (2, 13–16, 19). A decline in measured Ψ_Π can result from a simple passive concentration of solutes due to dehydration or due to net solute accumulation. The lowering of Ψ_Π by net solute accumulation is termed OA. OA has been considered a beneficial drought tolerance mechanism in some

field crop species (6, 9, 20, 22). Processes such as cell expansion are dependent on the influx of water driven by the maintenance of lower Ψ_Π by net solute increase in the expanding cell. The lowering of Ψ_Π by OA also minimizes the opportunity for significant water loss to occur from leaf tissue.

Passive concentration of solutes due to dehydration can arise from a decrease in WC per unit DW, reduced leaf tissue volume due to cellular shrinkage, change in leaf tissue elasticity, or relative partitioning of water between the symplastic and apoplastic fractions. Valid estimates of OA can only be made on measured Ψ_Π corrected to full hydration or Ψ_Π at zero turgor to remove the effect of dehydration. A decrease in Ψ_Π at full hydration can result from concentration of solutes due to reduced osmotic volume as the result of an increase in insoluble polymer accumulation, such as starch, which is reflected in the TW/DW ratio. The decline in Ψ_Π at full hydration due to change in TW/DW is not considered active OA. The occurrence of active OA can be established only if a net increase in solute concentration occurs at full hydration.

Several reports of diurnal changes in Ψ_Π in sorghum exist (1, 3, 4, 18). However, most of these studies did not quantitatively differentiate between net solute accumulation and passive concentration effects on lowering Ψ_Π. In most studies in which Ψ_Π is measured with a psychrometer (by freezing and thawing), Ψ_Π is not corrected for dilution of symplastic solutes by apoplastic water, resulting in underestimation of OA. If OA by net solute accumulation is truly a beneficial mechanism, then accurate estimates of when it occurs and the extent of change are important considerations. The objective of this experiment was to determine the relative contribution of each type of mechanism involved in diurnal Ψ_Π changes in grain sorghum leaves in response to water stress.

MATERIALS AND METHODS

Sorghum (*Sorghum bicolor* L. Moench cv ATx623 × RTx430) was planted in the field on May 29, 1985, in a randomized complete block design using two water supply treatments and four replications. Each plot consisted of 10 rows, spaced 0.68 m apart and 15 m long. Final plant density was 18 to 20 plants m^{-2}. The field site has a loamy fine sand soil texture (fine, loamy mixed thermic family of aridic Paluestalf) approximately 2 m deep. Nitrogen fertilizer was applied before planting and 35 d after emergence at the rate of 100 kg N/ha at each application. Water was applied with an automated surface trickle irrigation system using a 3-d fre-

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[1] Abbreviations: Ψ_w, water potential (MPa); Ψ_Π, osmotic potential (MPa); Ψ_p, turgor potential (MPa); Ψ_Π^{100}, Ψ_Π at full hydration (MPa); RWC, relative water content (%); OA, osmotic adjustment (MPa); ϵ, modulus of elasticity (MPa); TW, turgid weight (g); DW, dry weight (g); WC, water content (g).

Figure 1. Diurnal changes in leaf Ψ_w and Ψ_{Π} of the uppermost fully expanded leaves of sorghum at different growth stages for well-watered and stressed treatments. Ψ_w: ●, well watered; ■, stressed; Ψ_{Π}: ○, well watered; □, stressed. Each data point represents pooled data of 2-d measurements for each growth stage. SD averaged ± 0.12 for all measurements ($n = 8$). CDT, Central daylight time.
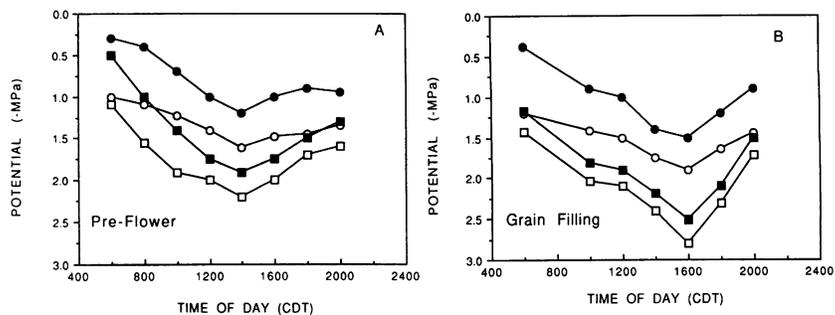
quency schedule. Two water supply treatments were established. The first treatment represented replacement of 100% of the pan evaporation (measured at the site) during the previous 3 d. The second treatment received no supplemental irrigation and reflected the dryland condition (stressed). The water supply treatments were started 35 d after emergence. A total of 85 mm of rainfall in several events occurred throughout the growing season.

Measurements of leaf Ψ_w, Ψ_{Π}, and RWC were made on the middle portion of expanding and uppermost fully expanded leaves before flowering and on the first leaf below the flag leaf during grain filling. Diurnal measurements were made at 2-h intervals between 0600 and 2000 h on the well-watered and stressed treatments. The measurements were taken twice at 14-d intervals during each growth stage.

Ψ_w and Ψ_{Π} were determined using leaf cutter psychrometers (J.R.D. Merrill Speciality Co., Logan, UT). Equilibration times of 4 h for Ψ_w and 1 h for Ψ_{Π} (after freezing in liquid N_2) at 30°C were used. Apoplastic WC was estimated using pressure-volume relationships. For the construction of a pressure-volume curve, the uppermost fully expanded leaves were sampled from well-watered and water-stressed treatments. After fresh weight and blade area were measured, the leaves were placed in a beaker with their cut ends submerged in water and left for 12 h. The fully TW of the leaves was then recorded, and the blade was allowed to slowly dry at 20°C and 50% RH. Water loss was determined by monitoring the change in fresh weight with time during a 4-h period. Leaf discs were removed at each weight measurement time, and Ψ_w was determined. Apoplastic WC was calculated by extrapolation of the linear region of the relationship between $1/\Psi_w$ versus RWC. Apoplastic WC was used to correct the dilution effect on the measured Ψ_{Π} as described by Jones and Rawson

(11). Turgor potential was calculated as the difference between Ψ_{Π} (corrected) and Ψ_w.

RWC was determined on the same leaf tissue used for Ψ_w measurement using the method described by Barrs and Weatherley (5). To minimize the cut surface effect and solute leakage, approximately 50 cm^2 of the middle portion of the leaf blade was used.

Ψ_{Π} at full hydration (100% RWC) was calculated using the following formula by Wilson et al. (21):

$$\Psi_{\Pi}{}^{100} = \Psi_{\Pi} \times \frac{RWC - B}{100 - B} \qquad (1)$$

where B is the apoplastic water fraction. Diurnal changes in Ψ_{Π} ($\Delta\Psi_{\Pi}$) and $\Psi_{\Pi}{}^{100}$ ($\Delta\Psi_{\Pi}{}^{100}$) were calculated as the difference in Ψ_{Π} and $\Psi_{\Pi}{}^{100}$ between dawn (dn) and the various sampling times during the day (dy).

$$\Delta\Psi_{\Pi} = (\Psi_{\Pi})^{dn} - (\Psi_{\Pi})^{dy} \qquad (2)$$

$$\Delta\Psi_{\Pi}{}^{100} = (\Psi_{\Pi}{}^{100})^{dn} - (\Psi_{\Pi}{}^{100})^{dy} \qquad (3)$$
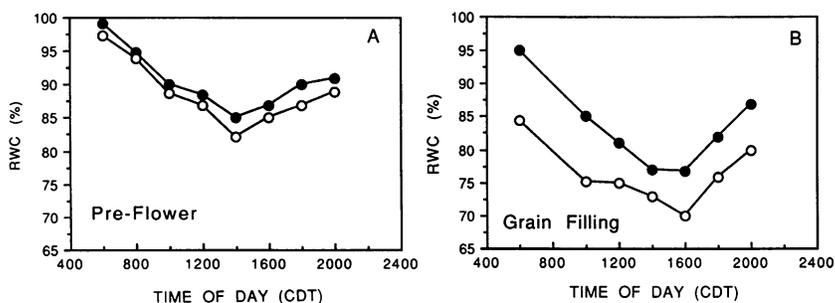
The contribution of dehydration to change in Ψ_{Π} ($\Psi_{\Pi}{}^d$) was calculated using the following formula:

$$\Psi_{\Pi}{}^d = \Delta\Psi_{\Pi} - \Delta\Psi_{\Pi}{}^{100} \qquad (4)$$

Solute concentration resulting from changes in nonosmotic volume due to insoluble polymer accumulation at full hydration (td) was estimated from changes in the TW/DW ratio between dawn (dn) and various times during the day (dy).

$$\Psi_{\Pi}{}^{td} = \frac{(TW/DW)^{dn} - (TW/DW)^{dy}}{(TW/DW)^{dn}} \times \Delta\Psi_{\Pi}{}^{100} \qquad (5)$$

Figure 2. Diurnal trends in RWC of the uppermost fully expanded leaves of sorghum at different growth stages for well-watered (●) and stressed (○) treatments. Each data point represents pooled data of 2-d measurements for each growth stage. SD averaged ± 2.5 for all measurements ($n = 8$). CDT, Central daylight time.
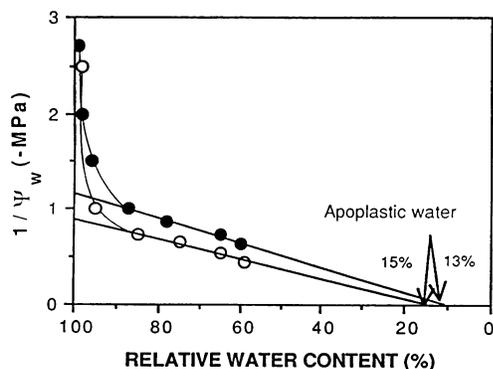
Figure 3. Pressure-volume curve for the uppermost fully expanded sorghum leaves for well-watered (●) and stressed (○) treatments before flowering.

The contribution of net solute accumulation ($\Psi_\pi{}^{net}$) to change in Ψ_π was calculated as:

$$\Psi_\pi{}^{net} = \Delta\psi_\pi{}^{100} - \Delta\Psi_\pi{}^{td} \qquad (6)$$

The bulk volumetric ϵ was calculated using the following formula:

$$\epsilon = \frac{\Delta\Psi_p}{\Delta RWC} \qquad (7)$$

where $\Delta\Psi_p$ is the change in Ψ_p and ΔRWC is the change in RWC on diurnal basis. All data were subjected to analysis of variance and regression analysis using the 1985 Statistical Analysis System (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

No significant differences ($P < 0.05$) in water relations parameters were observed between the 2 d of diurnal measurements within each growth stage. Water relations parameters were also not significantly different ($P < 0.05$) between the uppermost fully expanded and the expanding leaves. Therefore, the diurnal data presented are from the uppermost fully expanded leaves representing pooled data from 2 d of measurements for each growth stage.

Diurnal changes in Ψ_w and Ψ_π during preflowering and grain-filling periods are shown in Figure 1. Within each growth stage, Ψ_w and Ψ_π changed in response to diurnal evaporative demand as reported by others for sorghum (1, 3,

18). Ψ_w declined to a minimum of -1.2 MPa ($\Psi_\pi = -1.6$ MPa) in the well-watered treatment and to -1.9 MPa ($\Psi_\pi = -2.2$ MPa) in the stressed treatment before flowering. During grain filling, the minimum Ψ_w was -1.5 MPa ($\Psi_\pi = -1.9$ MPa) for well-watered and -2.5 MPa ($\Psi_\pi = -2.8$ MPa) for the stressed treatment. The magnitude of diurnal change in Ψ_π was greater in the stressed treatment than the well-watered treatment. The stressed treatment also had a greater diurnal change in both Ψ_π and Ψ_w during grain filling than the preflowering growth stage.

The diurnal trend in RWC was similar to that of Ψ_w and Ψ_π (Fig. 2). Before flowering, the minimum RWC was 85% for well-watered and 82% for the stressed treatment. After flowering, the minimum RWC was 77% for well-watered and 70% for the stressed treatment. The relationship between Ψ_w and RWC was altered because of water stress. This is evident from the pressure-volume curve shown in Figure 3. At comparable RWC, the stressed treatment developed a lower Ψ_w than the well-watered treatment. The relationship of RWC with Ψ_π indicates that the stressed treatment had a lower Ψ_π at 100% RWC than the well-watered treatment (data not shown). This suggests net solute accumulation in the stressed treatment.

The relationship of Ψ_π and $\Psi_\pi{}^{100}$ with Ψ_w for different growth stages are depicted in Figure 4. At both growth stages Ψ_π and $\Psi_\pi{}^{100}$ declined with decreased Ψ_w. The difference in slope between Ψ_π (not corrected for dehydration) and $\Psi_\pi{}^{100}$ indicates the extent of the dehydration effect on Ψ_π changes. The fact that $\Psi_\pi{}^{100}$ declined indicates that the solute concentration increased because of mechanisms other than dehydration. The occurrence and the extent of OA due to net solute accumulation can only be established after partitioning and removing the effects of passive concentration due to dehydration and changes in nonosmotic volume (decreased TW/DW ratio) from the total diurnal $\Delta\Psi_\pi$. The diurnal changes in Ψ_π due to dehydration, net solute accumulation, and change in TW/DW were partitioned by the methods described (Eqs. 2–6) and depicted in Figure 5. Before flowering, the diurnal $\Delta\Psi_\pi$ was 0.53 MPa for well-watered and 1.1 MPa for the water-stressed leaves. During grain filling, diurnal $\Delta\Psi_\pi$ was 0.70 MPa for well-watered and 1.4 MPa for the stressed treatment. Within both growth stages and across all water supply treatments, dehydration and net solute accumulation were the major mechanisms involved in diurnal $\Delta\Psi_\pi$. The change in Ψ_π due to changes in nonosmotic volume was minimal, as expected for C_4 leaves that do not store appreciable quantities of starch during the day.
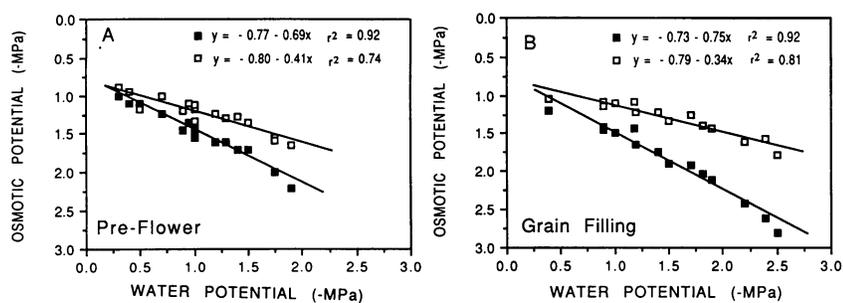

Figure 4. Relation between measured Ψ_π (■) and Ψ_π at 100% RWC (□) with Ψ_w for the uppermost fully expanded leaves of sorghum at different growth stages.
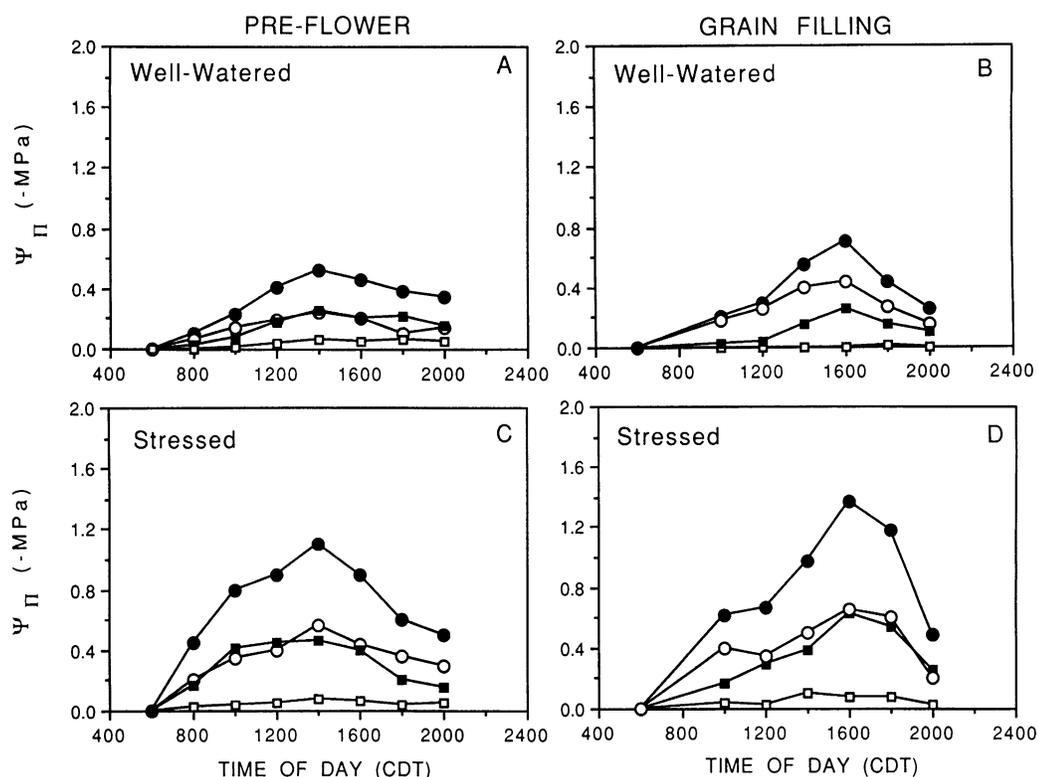
Figure 5. Total diurnal $\Delta\Psi_\Pi$ changes (●) and change in Ψ_Π due to dehydration (○), net solute accumulation (■), and TW/DW ratio (□) of the uppermost fully expanded leaves of sorghum for well-watered and stressed treatments at different growth stages. Data points represent pooled data of 2-d measurements from each growth period. CDT, Central daylight time.

Before flowering, dehydration accounted for 45% (0.24 MPa) of the diurnal $\Delta\Psi_\Pi$ under well-watered conditions and 50% (0.55 MPa) of the change in water-stressed leaves (Fig. 5). During grain filling, dehydration was responsible for 63% (0.44 MPa) of the diurnal $\Delta\Psi_\Pi$ under well-watered conditions and 47% (0.66 MPa) of the change in the stressed treatment. The contribution of dehydration to diurnal $\Delta\Psi_\Pi$ was 18% greater during grain filling than during the preflowering growth stage under well-watered conditions. No significant difference was observed in the relative contribution of dehydration to diurnal $\Delta\Psi_\Pi$ between the two growth stages in water-stressed leaves.

The observed diurnal $\Delta\Psi_\Pi$ due to dehydration (Fig. 5) can occur because of a decrease in tissue volume, change in leaf

tissue elasticity, or partitioning of water between the apoplast and symplast. At both growth stages, tissue volume decreased (indicated by WC/DW) with decreased RWC as shown in Figure 6. This indicates passive concentration of solutes as cellular volume decreases due to dehydration. The relationship of ϵ with Ψ_w for both pre- and postflowering growth stages is depicted in Figure 7. At both growth stages and water supply levels, ϵ remained essentially constant as Ψ_w decreased. This indicates that water stress did not change the elastic properties of the leaf tissue. The fact that water stress did not change the elastic properties of the leaf tissue does not mean that cellular shrinkage due to water loss did not occur, but it suggests that the rate of cellular shrinkage per unit water loss

Figure 6. Relation between WC/DW and RWC for the uppermost fully expanded leaves of sorghum for well-watered (●) and stressed (○) treatments at different growth stages. Data points represent pooled data of 2-d diurnal measurements from each growth period. Lines are fitted by eye.
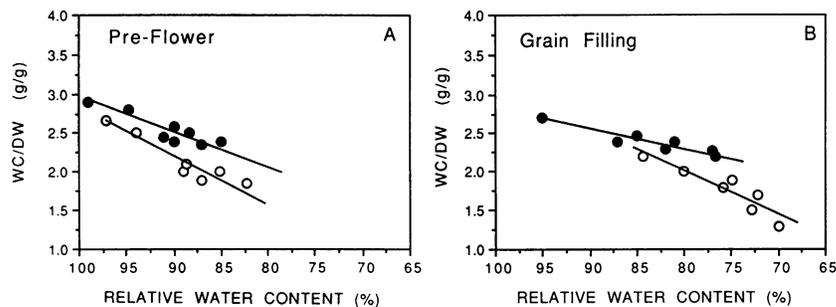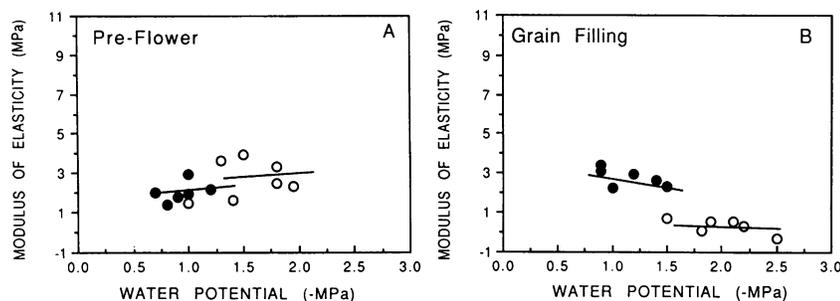
Figure 7. Relation between ϵ and Ψ_w for the uppermost fully expanded leaves of sorghum for well-watered (●) and stressed (O) treatments at different growth stages. Data points represent pooled data of 2-d diurnal measurements from each growth period. Lines are fitted by eye.

occurred at the same rate with increased water stress. For a given amount of water loss, an elastic cell wall would tend to shrink more than a rigid cell, resulting in concentration of solutes due to more tissue mass per unit volume.

At low Ψ_w, the partitioning of water into symplast and apoplast water fractions can also contribute to passive concentration of solutes. For a given amount of solutes, leaf tissue with a greater apoplast water fraction exhibits a more rapid concentration of solutes as RWC decreases (7, 17). Water stress did not significantly ($P < 0.05$) affect the RWC between the symplast and apoplast (Fig. 3), suggesting that the ratio of cell wall volume to cell volume was not significantly altered by water stress. The apoplastic water fraction was 13% for well-watered and 15% for stressed treatments. Therefore, the relative partitioning of water in the symplastic and apoplastic fraction did not contribute to the observed diurnal $\Delta\Psi_{II}$. Flower et al. (8) also found no significant differences in apoplastic water fraction between well-watered and stressed sorghum leaves. Because water stress did not affect leaf issue elasticity or partitioning of water fraction between symplast and apoplast, the observed diurnal $\Delta\Psi_{II}$ due to dehydration was mainly the result of decreased cellular volume (decreased WC/DW ratio). Jones and Turner (12) and Fereres et al. (7) indicated that leaf tissue elasticity is not associated with change in Ψ_{II} in sorghum.

The relative contribution of changes in nonosmotic volume as indicated by changes in TW/DW ratio to diurnal $\Delta\Psi_{II}$ was <8% at both growth stages and water supply treatments (Fig. 5). The decrease in TW/DW with decreased Ψ_w (Fig. 8) indicates a decrease in the osmotic volume, which resulted in

passive concentration of solutes at full hydration. Because the contribution of soluble sugars to the DW of sorghum leaves is minimal (17) and structural changes do not occur diurnally, we assume that the observed decrease in TW/DW could be due to increased insoluble polymers. The most logical insoluble polymer to accumulate diurnally is starch. However, sorghum being a C_4 plant, large accumulations of starch are unlikely in comparison with C_3 plants such as cotton (2).

The observed decrease in Ψ_{II}^{100} with decreased Ψ_w (Fig. 4) was mainly due to net solute accumulation. Before flowering, net solute accumulation due to OA accounted for 49% (0.26 MPa) of the diurnal $\Delta\Psi_{II}$ for well-watered and 42% (0.46 MPa) of the changes for the stressed treatment. During grain filling, net solute accumulation accounted for 37% (0.26 MPa) of the diurnal $\Delta\Psi_{II}$ for the well-watered leaves and 45% (0.63 MPa) of the changes in water-stressed leaves. The results indicate the occurrence of active OA due to net solute accumulation in sorghum as reported by others (1, 18). Acevedo et al. (1) indicated involvement of recent photosynthate in diurnal OA. Accumulation of soluble sugar and organic acids have been reported in water-stressed sorghum leaves (10).

The various possible mechanisms of diurnal Ψ_{II} changes in sorghum leaves in response to water stress have been examined. Results from this study indicated that reduced cellular volume associated with dehydration and net solute accumulation are the mechanisms involved in diurnal changes in Ψ_{II} in sorghum. The relative contribution of changes in nonosmotic volume (decreased TW/DW) to change in Ψ_{II} was minimal. Water stress did not affect leaf tissue elasticity or partitioning of water between the symplasm and apoplasm.
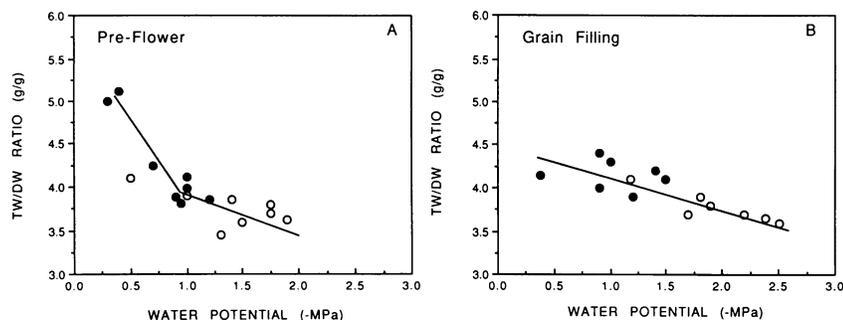

Figure 8. Relation between TW/DW and Ψ_w for the uppermost fully expanded leaves of sorghum for well-watered (●) and stressed (O) treatments at different growth stages. Data points represent pooled data of 2-d diurnal measurements from each growth period. Lines are fitted by eye.

The results from this study indicate that active diurnal OA in response to water stress occurred in sorghum leaves but represented <50% of the total change in Ψ_{Π}.

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