Genotypic Variation of Osmotic Adjustment, Water-use and Transpiration Efficiency among Closely Related Wheat Lines

QINGWU XUE1, B. A. STEWART2, MARK D. LAZAR1, GIOVANNI PICCINNI3, and CLAY D. SALISBURY1

1Texas AgriLife Research and Extension Center at Amarillo, Amarillo, Texas, USA
2Dryland Agriculture Institute, West Texas A&M University, Canyon, Texas, USA
3Monsanto Company, Chesterfield, Missouri, USA

Osmotic adjustment (OA) is an important adaptive response to water stress (WS) in wheat (Triticum aestivum L.). The objectives of this study were to determine the relationships between (1) OA and drought susceptibility, (2) OA and biomass, and (3) biomass, water-use efficiency (WUE), and transpiration efficiency (TE) in two cultivars and three closely related lines. Water stress treatments were imposed by polyethylene glycol (PEG)-8000 in nutrient solutions. Under WS, drought-resistant genotypes had higher OA than drought-susceptible ones. Comparing the two cultivars, higher OA may contribute to higher biomass. Among the three closely related lines, there was no correlation between OA and biomass and the genotype with higher OA having lower biomass. Under WS, biomass had a strong correlation with evapotranspiration (ET) and transpiration (T), but a weak correlation with WUE and TE. The results indicated that selecting traits related to effective use of water might be more important than traits related to WUE or TE.

KEYWORDS genotype, wheat, water relations, osmotic adjustment, drought resistance, transpiration efficiency

ABBREVIATIONS ($\Psi_w$) leaf water potential; ($\Psi_\pi$) leaf osmotic potential; (ET) evapotranspiration; (OA) osmotic adjustment; (PEG) polyethylene glycol; (RWC) leaf relative

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Address correspondence to Qingwu Xue at Texas AgriLife Research and Extension Center at Amarillo, 6500 Amarillo Blvd. W., Amarillo, TX 79106, USA. E-mail: Qxue@ag.tamu.edu
INTRODUCTION

Wheat is widely grown under both dryland and irrigated conditions for grain and winter cattle forage in the U.S. Southern High Plains (i.e., Texas High Plains, the Oklahoma Panhandle, parts of eastern New Mexico, and southwestern Kansas) (Winter & Musick 1993; Musick et al. 1994; Howell et al. 1995). The area has a semi-arid climate with annual precipitation that ranges from 380 mm in the southwest to 580 mm in the northeast, with averages of about 480 mm. The growing-season precipitation for wheat production averages about 250 mm or approximately one third of evapotranspiration (ET) requirement for high yields (Musick et al. 1994). As a result, drought stress is the most common factor reducing grain yield in the area (Johnson, Nguyen, & Croy 1984; Schonfeld et al. 1988; Musick et al. 1994; Xue et al. 2006). In the Texas High Plains, dryland wheat yield mostly ranges from 1,000 to 2,000 kg ha$^{-1}$. For irrigated wheat, yield is in the range of 3,000–8,000 kg ha$^{-1}$ (Musick et al. 1994; Howell et al. 1995; Jones & Popham 1997; Xue et al. 2006). The irrigation water supply for irrigated wheat in this area is primarily from the Ogallala aquifer. With declining water supplies from the aquifer and increasing energy costs, application of less irrigation water than the plants require for high yield (i.e., deficit irrigation) will be a common practice for irrigated wheat production. Because drought stress is inevitable under both dryland and deficit irrigation conditions, adoption of wheat cultivars with more drought resistance is critical for sustainable wheat production in the U.S. Southern High Plains.

A better understanding of drought resistance mechanisms may lead to the development of wheat with more drought resistance (Johnson, Nguyen, & Croy 1984; Reynolds, Mujeeb-Kazi, & Sawkins 2005; Blum 2009). However, drought resistance is complex and systematic improvement has been difficult. One way of simplifying analysis of drought resistance is through examination of variability among closely related genotypes (Lazar, Salisbury, & Worrall 1995). Field experiments have shown that significant variation in drought resistance exists among a set of closely related wheat lines (Lazar, Salisbury, & Worrall 1995; Balota et al. 2008). The systematic characterization of differences in physiological responses to drought stress among closely related lines may lead to better understanding of the mechanism of their drought resistance.

Osmotic adjustment (OA) has been suggested as an important trait of drought resistance in many crop species (Morgan 1984; Santamaria, Ludlow, & Fukai 1990; Babu et al. 1999). The OA is a process where plant cells
accumulate solutes to respond to a decrease in water potential as water stress develops. The function of OA is to maintain cell turgor, high relative water content (RWC) in leaves, and physiological processes under water stress (Jones & Turner 1980; Morgan 1984; Blum 2005). Genotypic variation of OA has been found in wheat cultivars (Morgan, Hare, & Fletcher 1986; Morgan 1992). Studies have shown that there is a positive correlation between high OA and sustained yield or biomass under water-limited conditions across cultivars in different crops, including wheat (Blum 2005). Although the physiological responses in wheat cultivars under drought stress have been studied in the U.S. Southern High Plains (Johnson, Nguyen, & Croy 1984; Schonfeld et al. 1988; Winter, Musick, & Porter 1988), there is no information about OA among the closely related wheat lines. In addition, the relationship between OA and biomass production is largely unknown for these lines.

Under water-limited conditions, crop yield is determined by the biomass production as well as the proportion of biomass partitioning to grain (harvest index, HI). Biomass is associated with the amount of ET and water-use efficiency (WUE), the ratio of biomass, and ET (Passioura 1977; Ehdaie 1995; Richards et al. 2002). In general, biomass production is linearly related to plant transpiration (T), the major part of ET. Effective use of water (EUW), maximizing soil water uptake for T, can be very important to increase biomass under water stress (Musick et al. 1994; Blum 2009). Water-use efficiency is related to soil evaporation (E) and transpiration efficiency (TE), the ratio of biomass and T. Soil evaporation occurs mainly during the early growth stage when the soil surface is only partially covered by the plant canopy. As biomass production is increased at late development stages, evaporation will be reduced and the major portion of water extracted from the soil profile will be used for transpiration (Ehdaie 1995). The WUE is largely determined by TE, and plants with higher TE generally have higher WUE when E is low. Both EUW and WUE may be used as selection traits for improving wheat-biomass production under water-limited conditions (Reynolds, Mujeeb-Kazi, & Sawkins 2005). Blum (2009) emphasized that improving EUW and not WUE should be the target of crop yield improvement under drought stress. However, other studies suggested that WUE or TE can be used as a trait for selection of cultivars with high yield and biomass for environments where water is limited during grain filling (Richards et al. 2002; Reynolds & Tuberosa 2008). Genetic variation in WUE and TE at leaf level has been reported in the central Great Plains (Morgan et al. 1993; Xue et al. 2002). However, little is known about the genetic variation in water use (e.g., EUW), WUE, and TE among wheat cultivars in the U.S. Southern High Plains.

In this study, we conducted two pot experiments in a growth chamber using five wheat genotypes with known drought susceptibility. The objectives were to determine the relationships between (1) osmotic adjustment
Genotypic Variation of OA and Transpiration Efficiency

(261) and drought susceptibility, (2) OA and biomass accumulation, and (3) biomass, water-use efficiency (WUE), and transpiration efficiency (TE) among wheat genotypes under water stress.

MATERIALS AND METHODS

Plant Materials and Culture Conditions

Two pot experiments (Experiments I and II) were conducted in a walk-in environmental growth chamber (Environmental Growth Chambers, Chagrin Falls, OH, USA). Five wheat genotypes were selected for experimentation. These genotypes included two cultivars, TAM W-101 and Sturdy, and three closely related experimental lines, TX86A8072, TX86A6880, and TX86A5606. The three experimental lines are BC3-derived lines from a cross of a TAM-107 Sib (recurrent parent) with a germplasm line, Largo. They have the same pedigrees as TAM-105*4/Amigo*4//Largo, with a coefficient of parentage of 0.94 (Lazar et al. 1996). Field experiments (Lazar, Salisbury, & Worrall 1995) demonstrated that though the anthesis and maturity dates and grain-filling duration were similar among the genotypes, they differed significantly in yield and drought-susceptibility index (S) (Fischer & Maurer 1978) under drought stress. TX86A8072 had the highest yield and lowest S value, and TX86A5606 had the lowest yield and highest S value. Yield and S value of TX86A6880 were intermediate to those of TX86A8072 and TX86A5606. A different field study further confirmed that TX86A8072 was more drought resistant and produced higher yield than TX86A5606 under drought conditions (Balota et al. 2008). The two cultivars, TAM W-101 and Sturdy, have been identified as drought resistant and susceptible, respectively, in previous studies (Johnson, Nguyen, & Croy 1984; Schonfeld et al. 1988; Winter, Musick, & Porter 1988; Ritchie, Nguyen, & Holaday 1990).

Seeds were planted in soil in ice-cube trays. Each ice-cube tray had 16 cells, each one 5 × 3 × 3.5 cm. Eight seeds were planted in each cell and raised to the two-leaf stage (about one week after planting), and the seedlings were vernalized at about 8°C for 6 weeks. Then, after the soil was washed from the roots, the vernalized plants were transplanted into polyvinyl chloride (PVC) tubes (pots) with one plant per pot. The PVC pot was 100 cm tall, 5.2 cm in interior diameter, and 0.5 cm thick. Each pot was filled with 1.2 kg fritted clay (Absorb-N-Dry, Flatonia, TX, USA), a chemically inert soil matrix. Fritted clay has a dry bulk density of 0.67 g cm⁻³, a particle density of 2.5 g cm⁻³, and a total porosity of 0.73. After drainage from commonly used containers, the material holds 31% by volume of plant-available water and has an air-filled porosity of 28%, making it an excellent soil for plant growth (van Bavel, Lascano, & Wilson 1978). When filling the pots, limestone (2.54 g) and diammonium phosphate (5.29 g) were mixed with the fritted clay. At the beginning of the experiment, pot capacity...
(PC) was determined by thoroughly watering the pots with half-strength Hoagland's solution and then weighing them when drainage ceased. Prior to transplanting, the pots were watered again to the determined PC. Then, during the experiment, plants were watered with Hoagland's solution twice a week until jointing, and every two days after jointing. Plants were grown to maturity in the growth chamber, where photosynthetically active radiation (PAR) intensity was maintained at 600 µmol m$^{-2}$ s$^{-1}$ at the canopy level. Photoperiod was 10 hours from transplanting to jointing (JT) (Feekes scale 6; Large 1954), then 14 hours thereafter. The temperature regime was 20°C/15°C (day/night) from transplanting to JT, and 25°C/18°C after JT.

Experimental Design and Treatments

The experiments were designed as a split plot with water stress (WS) treatments as main plots and genotypes as subplots. In each main plot, five subplots were randomly arranged. Each experimental unit was a single plant in a PVC pot, having a combination of one genotype and one WS treatment level. For each experimental unit, there were sufficient (up to 20) replications for physiological measurements and biomass sampling at different growth stages. The WS treatments were imposed by adding PEG-8000 to the nutrient solution. In Experiment I, two PEG concentrations were used to produce solution water potentials of −0.5 MPa and −1.0 MPa. The water potential of Hoagland's solution without PEG was −0.08 MPa (Michel 1983). The PEG-induced water-stress treatments were imposed at tillering (TI) and JT (Feekes scale 3 and 6; Large 1954). Plants in WS treatments were watered with PEG solutions regularly until maturity. Control plants (0) were watered with nutrient solution without PEG. In total, there were five treatments in experiment I (0, −0.5 MPa, and −1.0 MPa at TI, and −0.5 MPa and −1.0 MPa at JT). In Experiment II, four PEG concentrations were used to produce solution water potentials of −0.25, −0.5, −0.75, and −1.0 MPa, and the stress treatments were imposed at TI stage. The PEG concentrations used to produce solution water potential of −0.25, −0.5, −0.75, and −1.0 MPa were 10, 20, 25, and 30 g PEG/100 ml water, respectively, based on an empirically derived equation proposed by Michel (1983).

Measurements

Leaf water potential ($\Psi_{W}$), osmotic potential ($\Psi_{\pi}$), and relative water content (RWC)

The leaf-water-relations parameters were measured once a week at 7, 8, and 9 weeks after WS started at TI stage in Experiment I. Twice on each sampling date, first in the morning before turning on the lights and then 4 h after lighting, three leaf-tissue samples were collected per treatment
combination of WS and genotype. At each time, the three samples were taken from mature flag leaves of three different plants (pots), such that a total of six plants (pots) were sampled per day. Leaf-water potential ($\Psi_w$) and osmotic potential ($\Psi_\pi$) were measured using model 76-1A leaf cutter thermocouple psychrometers (J.R.D. Merrill Specialty Equipment, Logan, Utah). A 5-mm-diameter leaf disk was taken from the midpoint along the length of the leaf blade and placed in a thermocouple psychrometer. The $\Psi_w$ values were obtained after the psychrometers were equilibrated in a water bath at 25°C for 4 h. The psychrometers with samples were then frozen in liquid nitrogen for 10 min and thawed. The osmotic potential was measured after temperature equilibration in a water bath for 1 h at 25°C (Morgan 1980). Before measuring the water potential and osmotic potential, psychrometers were calibrated using standard NaCl solutions (0, 0.1, 0.2, 0.5, and 1.0 Mol L$^{-1}$), and a linear relationship between solution-water potential and psychrometer-output voltage was obtained (Morgan 1980; Brown & Osterhuis 1992).

After taking a sample for measuring $\Psi_w$ and $\Psi_\pi$, another sample was taken from the same leaf to measure RWC (Slatyer & Barrs 1965; Schonfeld et al. 1988; Turner et al. 2007). The samples were weighed to determine the fresh weight (FW) and then placed in distilled water for 6–8 h to determine the turgid weight (TW). After determining the TW, samples were oven-dried at 60°C for two days to determine dry weight (DW). The RWC was calculated as:

$$RWC = 100 \times \frac{(FW - DW)}{(TW - DW)} \quad [1]$$

Leaf osmotic adjustment for each genotype was determined by the linear regression of leaf RWC on $\Psi_\pi$ and the RWC values at $\Psi_\pi$ of $-2.5$ MPa (RWC$_{-2.5}$). The RWC$_{-2.5}$ values were calculated using the regression equation of RWC and $\Psi_\pi$. The greater slope of the regression of RWC and $\Psi_\pi$, and higher RWC$_{-2.5}$ value indicate higher OA (Morgan 1992).

**AVERAGE BIOMASS, GROWTH RATE (GR), EVAPOTRANSPIRATION (ET), TRANSPIRATION (T), WATER-USE EFFICIENCY (WUE), AND TRANSPIRATION EFFICIENCY (TE)**

At anthesis and physiological maturity (Feekes scale 10.5–11), aboveground plant samples were harvested to determine biomass. Samples were oven dried at 60°C for at least two days, then weighed. Based on biomass, the mean growth rate (GR) between anthesis and maturity was calculated using the equations of Radford (1967).

For each plant in each treatment, ET was calculated by weighing the PVC pots when they were watered by nutrient solutions as described above. The ET was defined as the difference between the weight at pot capacity and
at time of watering. The total ET was determined by accumulating the ET during the entire experiment (from transplanting to maturity). For each WS treatment, five pots without plants were selected and weighed for determining soil evaporation (E). The plant transpiration (T) was calculated by the difference between ET and E. The total T during the entire experiment was determined similarly to ET. The WUE and TE were calculated as the ratio of biomass to total ET and T, respectively (Stewart & Steiner 1990; Richards et al. 2002).

Statistical Analysis

Statistical analysis was conducted using SAS software (SAS Institute 2008). For analysis of variance, the general linear model (GLM) procedure was used to determine the differences among treatments, genotypes, and their interactions. When overall statistical significance ($P < 0.05$) was found, LSD tests were used to compare the means (Neter, Wasserman, & Kutner 1990). Relationships between RWC, $\Psi_w$, and $\Psi_\pi$ were determined via linear regression. Logarithmic transformation in RWC, $\Psi_w$, and $\Psi_\pi$ has been shown to improve the linear regression (Morgan 1992) and was therefore used when appropriate. Analysis of covariance was performed to test differences among regression lines (Freund, Littell, & Spector 1986; Neter, Wasserman, & Kutner 1990). In addition, the SAS NLIN procedure was used to conduct nonlinear regression of biomass, GR, ET, and T on WS levels in Experiment II.

RESULTS

Leaf Water Relations and Osmotic Adjustment (OA)

The leaf $\Psi_w$, $\Psi_\pi$, and RWC from samples taken before turning on the lights in Experiment I, averaged across five genotypes, are shown in Figure 1. Compared to plants without WS, the PEG-induced water stress significantly reduced leaf $\Psi_w$, $\Psi_\pi$, and RWC. The leaf $\Psi_w$, $\Psi_\pi$, and RWC 4 h after lighting showed a similar trend among WS treatments (data not shown).

The leaf RWC decreased linearly as $\Psi_w$ and $\Psi_\pi$ decreased (Figures 2 and 3). There were significant differences ($P < 0.05$) in regressions of RWC on $\Psi_w$ across all five genotypes when WS was initiated at tillering. The drought-resistant cultivar (TAM W-101) had a smaller slope than the drought-susceptible one (Sturdy). Among the three closely related experimental lines, drought-resistant lines (TX86A8072 and TX86A6880) had smaller slopes than the drought-susceptible line (TX86A5606) (Figure 2). When comparing genotypes at the same level of low $\Psi_w$ (−2.5 MPa), TAM W-101, TX86A8072, and TX86A6880 had higher RWC (68%–70%) than TX86A5606 and Sturdy (62% and 58%). Therefore, drought-resistant genotypes had higher RWC than drought-susceptible ones under WS conditions. When WS started at jointing
FIGURE 1 Leaf water potential ($\Psi_w$) and osmotic potential ($\Psi_\pi$) (A), and relative water content (RWC) (B) before lighting in the morning at different water stress treatments. Among water stress treatments, values with the same uppercase letters were not significantly different at $P > 0.05$ for $\Psi_w$ or RWC, and values with the same lowercase letters were not significantly different at $P > 0.05$ for $\Psi_\pi$. (All $\Psi_w$, $\Psi_\pi$, and RWC data were collected in Experiment I, and each data point is a mean of five genotypes.)

stage, the differences in linear regressions of RWC on $\Psi_w$ were significant between TAM W-101 and Sturdy at $P < 0.10$, in which TAM W-101 had a smaller slope than Sturdy (Table 1).

The RWC values at $\Psi_\pi$ of $-2.5$ MPa (RWC$_{-2.5}$) have been used to estimate the OA in wheat genotypes, and plants with greater RWC$_{-2.5}$ usually had higher OA (Morgan 1983, 1992). Leaf RWC decreased linearly with decreasing $\Psi_\pi$ when WS started at both TI and JT stages (Figure 3).
FIGURE 2 Linear regressions of leaf relative water content (RWC) on water potential ($\Psi_w$) over genotypes under water stress started at tillering (TI) stage (A, B) and at jointing (JT) stage (C, D). Each data point is a mean of three leaves in each genotype.

TABLE 1 Regression Coefficients of Leaf Relative Water Content (RWC) on Leaf Water Potential ($\Psi_w$), and RWC Values at $\Psi_w$ of −2.5 MPa Among Five Genotypes Under Water Stress Started at Tillering (TI) and Jointing (JT) Stages

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Intercept</th>
<th>Slope</th>
<th>$R^2$</th>
<th>RWC at $\Psi_w$ of −2.5 MPa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Started at TI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAM W-101</td>
<td>98.10*</td>
<td>11.13*</td>
<td>0.62</td>
<td>70.28</td>
</tr>
<tr>
<td>TX86A8072</td>
<td>96.09</td>
<td>11.10*</td>
<td>0.80</td>
<td>68.34</td>
</tr>
<tr>
<td>TX86A6880</td>
<td>96.20</td>
<td>11.47*</td>
<td>0.66</td>
<td>67.53</td>
</tr>
<tr>
<td>TX86A5606</td>
<td>100.25</td>
<td>15.30</td>
<td>0.86</td>
<td>62.00</td>
</tr>
<tr>
<td>Sturdy</td>
<td>102.71</td>
<td>17.91</td>
<td>0.71</td>
<td>57.94</td>
</tr>
<tr>
<td>Started at JT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAM W-101</td>
<td>97.60*</td>
<td>9.09$^d$</td>
<td>0.79</td>
<td>74.88</td>
</tr>
<tr>
<td>TX86A8072</td>
<td>96.59</td>
<td>12.65</td>
<td>0.76</td>
<td>64.96</td>
</tr>
<tr>
<td>TX86A6880</td>
<td>98.64*</td>
<td>11.50</td>
<td>0.89</td>
<td>69.89</td>
</tr>
<tr>
<td>TX86A5606</td>
<td>94.93</td>
<td>10.46</td>
<td>0.67</td>
<td>68.78</td>
</tr>
<tr>
<td>Sturdy</td>
<td>96.25</td>
<td>12.61</td>
<td>0.71</td>
<td>64.73</td>
</tr>
</tbody>
</table>

$^d$Significantly different than Sturdy at $P < 0.10$ and $P < 0.05$, respectively.
Comparing linear regressions of RWC on $\Psi_\pi$ across genotypes when WS started at TI, TAM W-101, TX86A8072, and TX86A6880 had smaller slopes and lower intercepts than TX86A5606 and Sturdy. When WS started at JT stage, TAM W-101, TX86A8072, and TX86A5606 had smaller slopes and lower intercepts than Sturdy. When WS started at TI, RWC$_{-2.5}$ values for TAM W-101, TX86A8072, and TX86A6880 were > 70%, whereas RWC$_{-2.5}$ values for TX86A5606 and Sturdy were only about 65%. When WS started at JT, the RWC$_{-2.5}$ values were generally higher than 70%, and TAM W-101 still had higher RWC$_{-2.5}$ than Sturdy (Table 2). Therefore, TAM W-101, TX86A8072, and TX86A6880 maintained higher RWC than Sturdy and TX86A5606 under low osmotic potential and thus had higher OA.

Biomass, Evapotranspiration (ET), Transpiration (T), Water-Use Efficiency (WUE), and Transpiration Efficiency (TE)

**EXPERIMENT I**

There was a significant WS-by-genotype interaction ($P < 0.05$) for biomass at both anthesis and maturity; therefore, the biomass values among genotypes
TABLE 2 Regression Coefficients of Leaf Relative Water Content (RWC) on Leaf Osmotic Potential (Ψπ) and RWC Values at Ψπ of −2.5 MPa (RWC_{−2.5}) Among Five Genotypes Under Water Stress Started at Tillering (TI) and Jointing (JT) Stages

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Intercept</th>
<th>Slope</th>
<th>R²</th>
<th>RWC_{−2.5} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Started at TI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAM W-101</td>
<td>104.55*</td>
<td>10.88*</td>
<td>0.43</td>
<td>77.35</td>
</tr>
<tr>
<td>TX86A8072</td>
<td>105.41*</td>
<td>13.10*</td>
<td>0.69</td>
<td>72.66</td>
</tr>
<tr>
<td>TX86A6880</td>
<td>107.48</td>
<td>13.16*</td>
<td>0.62</td>
<td>74.58</td>
</tr>
<tr>
<td>TX86A5606</td>
<td>121.99</td>
<td>22.02</td>
<td>0.65</td>
<td>66.94</td>
</tr>
<tr>
<td>Sturdy</td>
<td>131.29</td>
<td>26.72</td>
<td>0.74</td>
<td>64.49</td>
</tr>
<tr>
<td><strong>Started at JT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAM W-101</td>
<td>106.66**</td>
<td>11.39**</td>
<td>0.65</td>
<td>78.19</td>
</tr>
<tr>
<td>TX86A8072</td>
<td>113.00*</td>
<td>16.82*</td>
<td>0.75</td>
<td>70.95</td>
</tr>
<tr>
<td>TX86A6880</td>
<td>117.23</td>
<td>17.39</td>
<td>0.85</td>
<td>73.77</td>
</tr>
<tr>
<td>TX86A5606</td>
<td>111.90</td>
<td>14.89*</td>
<td>0.68</td>
<td>74.68</td>
</tr>
<tr>
<td>Sturdy</td>
<td>121.38</td>
<td>20.25</td>
<td>0.79</td>
<td>70.77</td>
</tr>
</tbody>
</table>

§, *, **Significantly different than Sturdy at P < 0.10, P < 0.05, and P < 0.01, respectively.

are presented for each WS treatment (Table 3). For control plants, TAM W-101 had higher biomass than the other genotypes at anthesis. At maturity, the two cultivars (TAM W-101 and Sturdy) had higher biomass than the three closely related lines. However, there were no significant differences in biomass among the three closely related lines in control plants (Table 3). Under PEG-induced WS, biomass production was related to WS severity as well as timing. Plants at −0.5 MPa treatment had higher biomass than those at −1.0 MPa treatment. At the same WS level (either −0.5 or −1.0 MPa),

TABLE 3 Aboveground Biomass at Anthesis and Maturity in Five Genotypes at Different Water Stress Treatment Levels in Experiment I

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control</th>
<th>Started at TI</th>
<th>Started at JT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass at anthesis (g plant⁻¹)</td>
<td></td>
<td>−0.5</td>
<td>−1.0</td>
</tr>
<tr>
<td>TAM W-101</td>
<td>7.36 a</td>
<td>3.48 a</td>
<td>2.53 a</td>
</tr>
<tr>
<td>TX86A8072</td>
<td>4.02 b</td>
<td>1.97 c</td>
<td>1.49 bc</td>
</tr>
<tr>
<td>TX86A6880</td>
<td>4.56 b</td>
<td>2.96 ab</td>
<td>2.42 ab</td>
</tr>
<tr>
<td>TX86A5606</td>
<td>4.22 b</td>
<td>3.08 a</td>
<td>2.40 ab</td>
</tr>
<tr>
<td>Sturdy</td>
<td>5.08 b</td>
<td>2.12 bc</td>
<td>1.19 b</td>
</tr>
<tr>
<td>Biomass at maturity (g plant⁻¹)</td>
<td></td>
<td>−0.5</td>
<td>−1.0</td>
</tr>
<tr>
<td>TAM W-101</td>
<td>10.49 a</td>
<td>4.33 a</td>
<td>3.08 a</td>
</tr>
<tr>
<td>TX86A8072</td>
<td>6.43 b</td>
<td>2.85 c</td>
<td>2.14 b</td>
</tr>
<tr>
<td>TX86A6880</td>
<td>7.09 b</td>
<td>3.55 c</td>
<td>3.17 a</td>
</tr>
<tr>
<td>TX86A5606</td>
<td>7.02 b</td>
<td>3.78 ab</td>
<td>2.40 ab</td>
</tr>
<tr>
<td>Sturdy</td>
<td>9.98 a</td>
<td>3.52 bc</td>
<td>2.75 ab</td>
</tr>
</tbody>
</table>

In each column, at either anthesis or maturity, the values with the same letters were not significantly different (P > 0.05).
plants that developed water stress earlier (at TI stage) had lower biomass than those that developed WS later (at JT stage). The genotypic variation in biomass was also related to WS severity and timing. Early WS (at TI stage) resulted in greater genotypic differences in biomass at both anthesis and maturity as compared with late WS (at JT stage). Comparing the two cultivars, TAM W-101 generally had higher biomass than Sturdy in control plants. There were no significant differences (P > 0.05) in biomass among the three closely related lines in control plants. However, TX86A8072 had lower biomass than either TX86A6880 or TX86A5606 under WS (Table 3). The WS significantly reduced plant-growth rate between anthesis and maturity (GR), regardless of WS treatment levels. Genotypic differences in GR were also found among the genotypes, and Sturdy had a higher GR than the three closely related lines. However, there were no significant differences in GR among the three closely related lines (Figure 4).

The ET and T amounts were reduced greatly by PEG-induced WS. Without WS, the total ET ranged from 3.7 to 5.8 kg pot\(^{-1}\) and total T from 3.0 to 5.2 kg pot\(^{-1}\), depending on genotype. Under WS conditions, the ET was in the range of 0.8–1.8 kg pot\(^{-1}\), and T in the range of 0.6–1.5 kg pot\(^{-1}\) (Table 4). The genotypic variation in ET and T was related to WS levels. Without WS, the two cultivars (TAM W-101 and Sturdy) had much higher ET and T than two of the three closely related lines (TX86A8072 and TX86A5606). Although the genotypic difference in ET and T varied with WS levels, TAM W-101 and Sturdy consistently had higher ET and T than either TX86A8072 or TX86A5606. There were no significant differences (P > 0.05) in ET and T among TAM W-101, Sturdy, and TX86A6880, as well as among the three closely related lines in any WS treatment (Table 4).

There was no significant genotype-by-WS interaction (P > 0.05) for WUE and TE. However, there were significant differences (P < 0.05) in WUE and TE among WS levels. The WUE and TE (1.71 and 2.01 kg m\(^{-3}\)) were the lowest in plants without WS, the highest (3.22 and 4.23 kg m\(^{-3}\)) at the WS of \(-1.0\) MPa that was started at TI stage, and were in the middle (2.39–2.61 kg m\(^{-3}\) for WUE and 3.00–3.20 kg m\(^{-3}\) for TE) in the other three WS treatments (Figure 5). There were no significant differences (P > 0.05) in WUE and TE among genotypes under both control and WS conditions. The average WUE and TE were 2.46 and 3.11 kg m\(^{-3}\), respectively. Correlation analysis showed that plant biomass at maturity was highly correlated to ET (r = 0.87, P < 0.001) and T (r = 0.88, P < 0.001). However, the biomass had no correlation with either WUE or TE. Therefore, the genotypic variation in biomass at maturity was largely associated with ET or T under water stress in this experiment.

**Experiment II**

There was no significant WS-by-genotype interaction (P > 0.05) for biomass, growth rate (GR), ET, T, WUE, and TE in Experiment II. Therefore, means
Plant growth rate (GR) between anthesis and maturity as affected by water stress treatments (A) and genotypes (B) in experiment I. Among either water stress treatments or genotypes, values with same uppercase letters were not significantly different at $P > 0.05$.

across genotypes were used to describe the response of these variables to WS, and means across WS treatments were used to discuss genotypic differences. The response of biomass to WS was fitted well to a nonlinear function at both anthesis and maturity. Biomass decreased substantially from non-stress to the first level of WS, then continued to decrease, but at a slow rate, as WS increased (Figure 6A). The response of GR between anthesis and maturity to WS followed the same trend as biomass (Figure 6B). At anthesis, there were no significant differences ($P > 0.05$) in biomass among genotypes except for TX86A8072, which had the lowest biomass. At maturity, TAM W-101 had the highest biomass, TX86A8072 and TX86A6880 had the lowest,
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Evapotranspiration (ET, kg pot⁻¹)</th>
<th>Transpiration (T, kg pot⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Started at TI</td>
<td>Started at JT</td>
</tr>
<tr>
<td>TAM W-101</td>
<td>5.50 a</td>
<td>1.55 a</td>
</tr>
<tr>
<td>TX86A8072</td>
<td>3.80 b</td>
<td>1.22 b</td>
</tr>
<tr>
<td>TX86A6880</td>
<td>4.78 ab</td>
<td>1.26 b</td>
</tr>
<tr>
<td>TX86A5606</td>
<td>3.68 b</td>
<td>1.16 b</td>
</tr>
<tr>
<td>Sturdy</td>
<td>5.81 a</td>
<td>1.24 b</td>
</tr>
</tbody>
</table>

In each column, at either anthesis or maturity, the values with the same letters were not significantly different (P > 0.05).

**FIGURE 5** Plant water use efficiency (WUE) and transpiration efficiency (TE) as affected by water stress in experiment I. At different water stress treatments, values with the same uppercase and lowercase letters were not significantly different at P > 0.05 for WUE and TE, respectively.
FIGURE 6 Biomass at anthesis and maturity (A) and growth rate (GR) between anthesis and maturity averaged across genotypes as affected by water stress in experiment II. Significance of nonlinear regressions: biomass at anthesis ($R^2 = 0.73$, $P < 0.001$) and biomass at maturity ($R^2 = 0.77$, $P < 0.001$; GR: $R^2 = 0.65$, $P < 0.001$).

and TX86A5606 and Sturdy were in the middle (Figure 7A). There were significant differences ($P < 0.05$) in GR among genotypes under both control and WS, and TAM W-101 had the highest GR. Among the three closely related lines, TX86A6880 had lower GR than TX86A8072 and TX86A5606 averaged across WS treatments (Figure 7B).

The responses of ET and T to WS were similar to biomass and GR, and ET and T decreased nonlinearly as the WS level became more negative (Figure 8A). Consistent with Experiment I, ET and T were reduced considerably under PEG-induced water stress. Even the lowest level of WS
Among genotypes, values with the same uppercase letters were not significantly different at $P > 0.05$ for biomass at anthesis or GR, and values with the same lowercase letters were not significantly different at $P > 0.05$ for biomass at maturity.

$(-0.25 \text{ MPa})$ resulted in a 62% reduction in ET as compared with the control plants. The differences in ET and T among the WS treatments were smaller than the differences between control and any of the WS treatments (Figure 8A). Similar to Experiment I, WS had significant effects ($P < 0.01$) on WUE and TE, and plants without WS had the lowest WUE and TE. Among the WS treatments, plants at WS of $-0.25 \text{ MPa}$ had higher WUE and TE than the other treatments (Figure 8B). Averaged across WS levels, TAM W-101 plants had higher ET and T than the other genotypes. However, there were no differences ($P > 0.05$) in ET and T among the three closely related lines and Sturdy (Figure 9A). There were significant
FIGURE 8 Evapotranspiration (ET) and transpiration (T)(A) and water use efficiency (WUE) and transpiration efficiency (TE)(B) averaged across genotypes as affected by water stress in experiment II. At different water stress levels, values with same uppercase and lowercase letters were not significantly different at \( P > 0.05 \) for WUE and TE, respectively. Significance of nonlinear regressions: ET (\( R^2 = 0.92, P < 0.001 \)) and T (\( R^2 = 0.91, P < 0.001 \)).

differences (\( P < 0.05 \)) in WUE and TE among genotypes. TAM W-101, Sturdy, and TX86A5606 had higher WUE than TX86A8072, whereas TAM W-101 also had higher WUE than both TX86A8072 and TX86A6880. The genotypic variation in TE was smaller as compared with that in WUE; however, TX86A8072 still had lower TE than other genotypes. Among the three closely related lines, TX86A5606 consistently had higher WUE than TX86A8072 (Figure 9B). Consistent with Experiment I, biomass at maturity was strongly correlated with ET and T (\( r = 0.88 \) for both biomass vs. ET and biomass vs. T, \( P < 0.001 \)). The correlation between biomass and WUE, although significant, was weak (\( r = 0.27, P < 0.05 \)) compared with ET and T.
FIGURE 9 Evapotranspiration (ET) and transpiration (T) (A) and water use efficiency (WUE) and transpiration efficiency (TE) (B) among genotypes averaged across water stress levels in experiment II. Among the genotypes, values with same uppercase letters were not significantly different at \( P > 0.05 \) for ET and WUE, and with the same lowercase letters were not significantly different at \( P > 0.05 \) for T and TE.

DISCUSSION

The Relationship Between Osmotic Adjustment and Drought Susceptibility Among Closely Related Wheat Lines

The results of this study showed that there was a positive relationship between osmotic adjustment and drought resistance among the three closely related lines. The drought-resistant lines (TX86A8072 and TX86A6880) showed higher OA than the drought-susceptible line.
(TX86A5606) under PEG-induced water stress. Osmotic adjustment is an important adaptive response to water stress in several crops (Morgan, Hare, & Fletcher 1986; Santamaria, Ludlow, & Fukai 1990; Blum & Pnuel 1990; Blum 2005). Although field study indicated that TX86A8072 and TX86A6880 were more drought resistant and had higher yield than TX86A5606 under water stress (Lazar, Salisbury, & Worrall 1995), the study did not elucidate the physiological mechanisms associated with drought resistance among these closely related lines. The differences in OA among these lines found in this study explained why they responded differently under water stress conditions. The consequences of OA are maintenance of leaf hydration and turgor potential (Morgan 1984; Schonfeld et al. 1988). The drought-resistant genotypes (TAM W-101 and TX86A8072) had higher RWC than susceptible genotypes (TX86A5606 and Sturdy) under water stress in this study. Therefore, the drought-resistant genotypes with a higher OA are able to maintain higher RWC than drought-susceptible genotypes under water stress. With the higher RWC, the drought-resistant line (TX86A8072) may be able to maintain higher photosynthetic rate and stomatal conductance than the drought-susceptible line (TX86A5606) under water stress (Balota et al. 2008).

The greater OA may result in more root growth and more water extractions from deeper soil profile (Morgan & Condon 1986; Sharp et al. 2004). Thus, the greater OA in TX86A8072 may explain why this line had longer roots than TX86A5606 under water stress (Lazar et al. 1997). Lopes and Reynolds (2010) found that deeper rooting in wheat is related to lower canopy temperature, higher stomatal conductance, and higher yield under water stress. Among the three closely related lines, Balota et al. (2008) showed that TX86A8072 and TXA6880 consistently had lower canopy temperature than TX86A5606 in a three-year field study. Apparently there is a relationship between OA and canopy temperature among the three closely related lines, and greater OA resulted in a cooler canopy. This finding is important for field screening drought resistance in wheat since crop canopy temperature can be easily measured and may provide estimates of genetic differences in yield among genotypes under field conditions (Balota et al. 2008; Lopes & Reynolds 2010). In addition, OA may provide plants with other functions such as sustaining cellular membrane and protein function, and maintaining chloroplast volume and function (Zhang, Nguyen, & Blum 1999). Since the three wheat lines used in this study are genetically close (Lazar, Salisbury, & Worrall 1995; Lazar et al. 1996), the differences in OA among these lines provided opportunities for genetic analysis of OA and other physiological processes. These lines may be used to identify genes, quantitative trait loci, and molecular markers related to OA in wheat (Jackie Rudd, personal communications).
Genotypic Variation of OA and Transpiration Efficiency

The Relationship Between OA and Biomass Accumulation

The higher OA in drought-resistant genotypes is associated with more biomass accumulation under water stress in wheat and sorghum (Wright, Smith, & Morgan 1983; Morgan 1984; Santamaria, Ludlow, & Fukai 1990). Comparing the two cultivars in this study, TAM W-101 frequently had higher biomass than Sturdy at both anthesis and maturity in both experiments under water stress. In addition, TAM W-101 showed higher growth rate during grain filling in Experiment II. Although the differences in biomass among the three closely related lines were generally small, TX86A8072 had lower biomass than TX86A6880 and TX86A5606 in both experiments. Apparently there was no relationship between OA and biomass accumulation among the closely related lines. In fact, though the drought-resistant line (TX86A8072) had higher OA, it had lower biomass as compared to the drought-susceptible line (TX86A5606). In a field study, Balota et al. (2008) did not find any differences in biomass at maturity among the three closely related lines under either dryland or irrigated conditions.

Under water-limited conditions, crop yield is determined by the biomass production as well as the harvest index (HI), the proportion of biomass partitioning to grain (Richards et al. 2002; Blum 2009). It appears that the two drought-resistant genotypes, TAM W-101 and TX86A8072, may have different mechanisms for obtaining higher yield under water stress. TAM W-101 consistently produced more biomass than the other genotypes in the two experiments in this study. In a field study, TAM W-101 had higher grain yield and biomass than Sturdy but they had the same HI (Schonfeld et al. 1988). However, the higher yield of TX86A8072 compared to TX86A5606 under drought stress was a result of a higher HI (Balota et al. 2008). The higher OA in the drought-resistant genotype TX86A8072 likely helps to maintain higher HI during grain filling under water stress. Field studies in wheat and sorghum have shown that higher OA resulted in higher HI in the drought-resistant cultivars (Morgan & Condon 1986; Santamaria, Ludlow, & Fukai 1990; Fan et al. 2008). HI is determined by photosynthesis during grain filling and remobilization of pre-anthesis carbon reserve from stems (Richards et al. 2002; Xue et al. 2006). The difference in photosynthesis during grain filling between TX86A8072 and TX86A5606 was reported, and the drought-resistant line (TX86A8072) had higher photosynthetic rate than drought-susceptible line (TX86A5606) (Balota et al. 2008). However, the difference in the remobilization of carbon reserves between TX86A8072 and TX86A5606 still needs further investigation under water stress.

The Relationship Between Biomass, Water Use Efficiency, and Transpiration Efficiency

In this study, the positive correlation between biomass at maturity and WUE or TE was not significant (P > 0.05) in Experiment I. Although a positive
correlation between biomass and WUE was significant in Experiment II, the correlation was generally weak ($r = 0.27$, $P < 0.05$). In contrast, biomass was highly correlated to ET ($r = 0.87$, $P < 0.001$) and T ($r = 0.88$, $P < 0.001$) in both experiments. These results indicated that ET or T is important to biomass production in wheat under water stress conditions in the Southern High Plains (Musick et al. 1994; Xue et al. 2003). Under water-limited conditions, higher biomass can be achieved by either increased ET or by higher WUE or both (Richards et al. 2002; Reynolds, Mujeeb-Kazi, & Sawkins 2005; Blum 2009). Increasing ET and T is related to improving water uptake through effective use of soil water (EUW) (Blum 2009). Based on the results from this study, selecting traits related to EUW may be more important than the traits related to WUE or TE. In fact, Blum (2009) argued that effective use of soil water is a major target for yield improvement in water-limited environments.

The genotypic variation in WUE or TE was found in Experiment II in this study. Among the three closely related lines, both the WUE and TE values were significantly different, with TX86A8072 having lower WUE and TE than TX86A5606. The two cultivars (TAM W-101 and Sturdy) had higher WUE and TE values than two of the three closely related lines (TA86A8072 and TX86A6880). However, there were no differences in WUE and TE between the two cultivars, TAM W-101 and Sturdy. Apparently, the drought-resistant genotypes (TAM W-101 and TX86A8072) did not have higher WUE or TE than the drought-susceptible ones (TX86A5606 and Sturdy) in this study. Frequently in the literature, drought resistance has been reported to be associated with low WUE with more water use from soil profile under limited water supply, and high WUE has likely been the result of lower water use (Blum 2009). For example, Kobata, Okuno, and Yamamoto (1996) showed that drought-resistant rice cultivars did not have higher WUE than the drought-susceptible ones. Instead, the drought-resistant cultivars had high ability to maintain transpiration under water stress through a deep root system. In a field study in central Great Plains in wheat, Xue et al. (2002) showed that two higher yielding cultivars consistently had lower leaf-level WUE than two lower yielding cultivars under both dry and wet conditions.

CONCLUSION

The characterization of differences in physiological responses to drought stress among the closely related wheat lines provided an opportunity to better understand the mechanism of drought resistance. Differences in OA among the closely related lines found in this study explained why they responded differently under water stress in the field studies. Drought-resistant genotypes with higher OA were able to maintain higher leaf RWC under water stress. Therefore, identifying traits related to maintaining higher
plant water content is important for drought resistance under Southern High Plains environment. The relationship between OA and biomass accumulation was related to genotypes. Higher OA might contribute to higher biomass in two cultivars. However, there was no correlation between OA and biomass among the three closely related lines. Nevertheless, biomass under water stress was largely determined by ET and T. Although genotypic differences in WUE and TE were found among the genotypes used in this study, WUE or TE did not have a strong correlation to biomass as compared with ET or T. These results indicate that selecting traits related to effective use of soil water may be more important than selecting for WUE or TE.

REFERENCES


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