Physiological responses to water stress and yield of winter wheat cultivars differing in drought tolerance


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Abstract
Moderate to severe drought (water stress) at the reproductive stage is common in the U.S. Southern High Plains (SHP), where wheat is grown as a major winter crop. The objective of this study was to better understand the physiological basis of drought tolerance in elite wheat cultivars. A 2-yr glasshouse study was conducted using three cultivars (TAM 111, TAM 112 and TAM 304) and two water treatments (wet: adequate water and dry: water-limited). Overall, TAM 111 and TAM 112 used more water for cumulative evapotranspiration (ET) and had more tillers and greater root mass and shoot mass compared to TAM 304. In the dry treatment, TAM 112 had 67% and 81% more grain yield than TAM 111 and TAM 304, respectively. Water use efficiency for grain (WUEg) and water use efficiency for biomass (WUEbm) were also greater in TAM 112 compared to the other cultivars in the dry treatment. The flag leaves in TAM 112 at mid-grain filling stage (about 15 days after flowering) had lower stomatal conductance (Gs), intercellular CO₂ concentration (Ci), transpiration rate (Tr) and net photosynthetic rate (Pn), but higher photosynthetic water use efficiency (PWUE) than TAM 111 and TAM 304 under water stress. This study demonstrated a distinct role of gas exchange parameters in response to drought, and TAM 112 was more efficient than TAM 111 and TAM 304 in evolving physiological mechanisms to adapt to water stress.

KEYWORDS
gas exchange, heading, maturity, mid-grain filling, photosynthesis

1 | INTRODUCTION

Hard red winter wheat (Triticum aestivum L.), the dominant crop in the U.S. Southern High Plains (SHP), is grown in a wide range of water regimes and produces both grain and forage (Howell, Steiner, Schneider, & Evett, 1995; Musick, Jones, Stewart, & Dusek, 1994; Xue, Zhu, Musick, Stewart, & Dusek, 2006; Xue et al., 2014). Growing season precipitation for wheat production in the SHP averages about 250 mm, but the seasonal evapotranspiration (ET) for the same period ranges from 700 to 950 mm under adequate water supply (Musick et al., 1994). As such, high wheat yields have to be achieved by irrigation using groundwater. However, the Ogallala Aquifer, a primary source of groundwater in the area, is rapidly depleting due to a lower recharge than the withdrawal rate (Colaizzi, Gowda, Marek, & Porter, 2009; Roberts, Male, & Toombs, 2007). Hence, water supply and distribution are the main limiting factors to wheat production in the area, where plants are often exposed to extreme, if not moderate drought stress.

The term drought represents a large diversity of conditions where soil moisture is limiting and reduces plant growth, reproduction and yield (Blum, 2011). A decrease in photosynthesis and increase in leaf senescence associated with drought stress during the grain filling period of wheat result in yield reduction (Kobata, Palta, & Turner, 1992; Palta, Kobata, Turner, & Fillery, 1994; Zhang et al., 2003).
1998). Under water-limited conditions, use of more recent cultivars that have the ability to extract more water from the deeper soil profile to increase yield is a key strategy for wheat management (Thapa et al., 2017; Xue et al., 2014). Wheat breeding has greatly contributed to minimize the impact of drought and to increase yield and water use efficiency (WUE) under the condition of limited water supply (Foulkes, Scott, & Sylvester-Bradley, 2002; Richards et al., 2010; Zhang, Chen, Sun, Wang, & Shao, 2010). For example, regional cultivar trials in the Texas High Plains (THP) showed that newer cultivars such as TAM 111 (released in 2003) and TAM 112 (released in 2005) consistently had higher yields (15%-30%) than a historic check, TAM W-101 (released in 1969), under both dryland and irrigated conditions (Bean, 2011).

In Texas, TAM 111 and TAM 112 remain the leading cultivars planted, accounting for 17.6% and 6.9% of the state’s 2016 planted wheat acres, respectively (USDA-NASS, 2016). Several breeding programmes, in and outside of the United States, have been using these two cultivars as crossing materials to improve drought tolerance in wheat. TAM 304 (released in 2008) is a more recent wheat cultivar. Unlike TAM 111 and TAM 112, distinct tolerance to lodging and resistance to leaf rust (caused by Puccinia triticina Eriks.) make TAM 304 another popular cultivar in the Southern Great Plains (Rudd et al., 2015). Our previous results showed that more drought-tolerant genotypes were able to extract water from the deeper soil profile, and had distinct gene expression profiles, elevated abscisic acid (ABA) levels, more aboveground biomass and a cooler canopy temperature (Pradhan et al., 2014; Reddy et al., 2014; Thapa et al., 2017; Xue et al., 2014). However, little is known about the cultivar differences in terms of physiological responses, especially variations in gas exchange parameters under water stress.

We hypothesized that the adaptation of winter wheat cultivars under the semi-arid climatic environments of the Texas High Plains can be characterized using their physiological traits and response mechanisms under varying water regimes. The objective of this study was to understand the physiological basis of drought tolerance in the leading wheat cultivars, TAM 111, TAM 112 and TAM 304 under the conditions of adequate (wet) and limited (dry) water supply.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Glasshouse experiments were conducted in 2012 (2012–2013) and 2013 (2013–2014), at the Texas A&M AgriLife Research andExtension Center, Amarillo, Texas (35.19°N, 102.06°W). Three wheat cultivars, TAM 111, TAM 112 and TAM 304, were grown at two water treatments (wet and dry) and harvested at three growth stages (heading, mid-grain filling and physiological maturity). Heading was the stage when more than 50% plants produced full heads, and the time about 15 days after flowering was considered as mid-grain filling. Sampling or harvesting stages were considered as blocks, and each block included three replications with a completely randomized arrangement of cultivar and irrigation treatments (Randomized Complete Block Design, RCBD). TLC1 potting mixture (Sun Gro Horticulture Canada Ltd.) was used as growing medium for emergence of seedlings. After emergence, the seedlings were vernalized at 4°C for 7 weeks. Seedlings (about two-leaf stage) were transplanted (23 November 2012 and 12 November 2013) into 7.5-L plastic pots filled with Turface QuickDry, a calcined clay (bulk density ~0.60 g/cm³), then moved into the glasshouse. Five seedlings were transplanted into each pot. At 14 days after transplanting (DAT), thinning was performed leaving three healthy and normal sized seedlings per pot. There were four pots for each treatment totalling 24 pots in each replication and 72 pots (216 plants) in each block.

Before transplanting, “Osmocote Controlled Release fertilizer” (N-P-K = 19.0-2.6-10.0%, 100 ppm N) was thoroughly mixed in the soil and “Miracle-Gro Water Soluble All Purpose Plant Food” (N-P-K = 24.0-3.5-13.0%, 100 ppm N) was added in four intervals along with irrigation water during seedling establishment. To ensure accurate evapotranspiration measurements, drainage from pots was eliminated by lining them with a plastic sheet, and evaporation from pots was minimized using rubber mulch on the surface. Before starting the dry treatment (50 DAT; jointing stage), all the pots were regularly watered to 100% pot capacity (PC: ~42% volumetric water content). The PC was determined using the method explained by Xue, Stewart, Lazar, Piccinni, and Salisbury (2012). After the dry treatment started, at each watering (2–3 times a week), the wet treatment pots were brought back to 90% PC in both years, but the dry treatment pots were only brought back to 50% PC in 2012 and 40% PC in 2013. The day/night temperatures in the glasshouse were initially set to 18/10°C. After the start of the dry treatment, the temperatures were increased to 22/14°C and further increased to 26/18°C after heading (75 DAT); however, the environmental conditions inside the glasshouse fluctuated a little with outside weather conditions. The mean relative humidity in the glasshouse was about 56 ± 17, 54 ± 10 and 48 ± 12%, for the periods of transplanting to jointing, jointing to heading and heading to maturity, respectively. During the heading to flowering stage in February 2013, the heaters in the glasshouse stopped working, so the glasshouse temperature was close to the outside temperature (~0°C at night) for about a week. Some of the wheat plants, mainly tillers, were affected by this low temperature (cold stress), which is discussed later.

2.2 | Data collection

The physiological data were collected from the middle portion of the fully expanded flag leaves, of the primary shoot, at mid-grain filling stage. Leaf chlorophyll content was determined using a Konica Minolta SPAD 502 meter. The gas exchange parameters, that is photosynthetic rate (Pn, μmol m⁻² s⁻¹), stomatal conductance (Gs, mol m⁻² s⁻¹), intercellular CO₂ concentration (Ci, μmol/mol) and transpiration rate (Tr, mmol m⁻² s⁻¹) were measured with a Li-6400 portable photosynthesis system (Li-cor, Inc., Lincoln, NE, USA). Twelve measurements from each cultivar (i.e. 18 measurements from each water level) were taken in the morning, 11:00 hrs and repeated in
the afternoon, 16:00 hrs, Central Standard Time (CST). In 2012, leaves in the gas exchange chamber were subjected to saturated light conditions of 1800 μmol m⁻²s⁻¹ PAR, but in 2013, in order to more closely mimic the diurnal change in light conditions experienced in the field, plants were given 1500 μmol m⁻²s⁻¹ PAR in the morning and 1800 μmol m⁻²s⁻¹ PAR in the afternoon (i.e. in Amarillo, greatest light intensity occurs at about 14:00 hrs during grain filling). Physiological data were collected from the plants that were to be harvested at maturity. Photosynthetic water use efficiency (PWUE) was calculated by dividing Pn to Gs (Ahmadi & Siosemardeh, 2005; Saeidi & Abdoli, 2015).

Tiller number at heading stage, plant height at the mid-grain filling stage and cumulative ET throughout the experimental period were measured. Plant samples were harvested at heading, mid-grain filling and physiological maturity. Before each harvest, the final pot weight was recorded. Water used (cumulative evapotranspiration) was calculated as, ET = pot weight at planting – pot weight at harvesting + total water added between planting and harvesting (Thapa, Stewart, & Xue, 2017; Thapa, Stewart, Xue, & Chen, 2017). After harvesting, plant samples were separated into shoot and root systems. Roots were collected by washing the calcined clay on a mesh screen. Plant samples were oven-dried at 60°C for 3 days, and dry weight was measured. The shoot-to-root ratio (S:R) was calculated on a dry weight basis. At maturity, heads were threshed to determine grain yield. The water use efficiency for grain (WUEg) and water use efficiency for biomass (WUEbm) were calculated as the ratios of grain yield and aboveground biomass (AGB) to total ET, respectively (Musick et al., 1994; Thapa, Stewart, Xue, & Chen, 2017; Xue et al., 2014).

2.3 | Statistical analysis

Statistical analysis was performed using SAS 9.4 (SAS Institute, Inc., 2013). Pots with weak or unhealthy plants were not included in the statistical analysis. Analysis of variance (ANOVA) was conducted using the PROC MIXED procedure to evaluate the effects of main factors (year, cultivar and water) and interactions. Replication was considered a random effect, whereas year, cultivar and water were fixed effects. Mean values were compared using the least significant difference (LSD) and considered significantly different at the 5% level. Linear regression was performed using PROC REG in SAS 9.4.

3 | RESULTS

3.1 | Evapotranspiration (ET)

Cumulative ET at crop maturity was significantly (p < .05) affected by all main effects (year, cultivar and water) and all two-way interactions except year × water (p = .0585; Table 1). Differences in cumulative ET among cultivars were more evident in the wet treatment than in the dry treatment (Table 2). For example, averaged across the years, compared to TAM 111 (30.91 kg/pot) and TAM 112 (31.85 kg/pot), TAM 304 (27.50 kg/pot) had significantly lower cumulative ET in the wet treatment, whereas there was no cultivar difference in the dry treatment. In 2012, averaged across the water treatment, TAM 304 had a mean cumulative ET of 21.31 kg/pot, which was lower than that in TAM 111 (24.13 kg/pot) and TAM 112 (25.43 kg/pot). There was no cultivar difference in cumulative ET in 2013. The mean cumulative ET values were significantly higher in 2013 (23.62 kg/pot) than in 2012 (20.58 kg/pot), and in the wet treatment (30 kg/pot) than in the dry treatment (14.05 kg/pot; Table 2).

3.2 | Plant height and tiller number

Plant height was significantly (p < .05) affected by all main effects and all two-way interactions, except cultivar × water (p = .5708; Table 1). Plants were shortest in TAM 304 and TAM 112 in 2012 and 2013, respectively (Table 2). The mean plant height was greater in 2012 (82.9 cm) than in 2013 (64.5 cm), and in the wet treatment (83.3 cm) than in the dry treatment (64.1 cm). Tiller number was significantly (p < .05) affected by all main effects and the year × water interaction (Table 1). For both years, tiller number was lower in the dry treatment than in the wet. Averaged across the year and water treatments, tiller number was lower in TAM 304 (12.4 tillers/pot) than in TAM 111 (16.0 tillers/pot) or TAM 112 (16.8 tillers/pot) (data not shown).

3.3 | Shoot and root mass

Aboveground biomass (shoot) at heading was significantly (p < .05) affected by all main effects, except year (p = .0798) and all two-way interactions (Table 3). Aboveground biomass at mid-grain filling showed a significant (p < .05) difference due to all main effects and all two-way interactions, whereas at maturity, AGB was significantly (p < .05) influenced by all main effects and the year × cultivar interaction. In 2012, at all growth stages (HD, GF and MT), the mean shoot mass was lower in TAM 304 than that in TAM 111 and TAM 112 (Table 4). Although the mean AGB at GF was similar among the cultivars in 2013, TAM 304 had lower AGB than TAM 111 at HD and MT. There was no cultivar difference in shoot mass in both dry and wet treatments, but the mean shoot mass was reduced by 46%–58% from wet to dry treatments at different growth stages (Table 4).

Root mass at heading was significantly (p < .05) affected by all main effects and all two-way interactions (Table 3). At mid-grain filling, root mass was significantly (p < .05) affected by all main effects and the cultivar × water interaction. At maturity, the significant (p < .05) difference was due to all main effects and all two-way interactions, except the year × water (p = .7642). Averaged across the water treatments, the root mass showed a similar trend as of shoot mass in both years, which was lower in TAM 304 than in TAM 111 and TAM 112 (Table 4). Averaged across the years, the cultivar difference in root mass was more evident in the wet treatment than in the dry treatment. For example, the mean root mass in the wet treatment was lower for TAM 304 than for TAM 111 and
The shoot-to-root ratio (S:R) was significantly \( (p < .05) \) affected by the main effect of year and all two-way interactions at HD, and by all main effects and all two-way interactions at GF and MT. A three-way interaction of year \times water \times cultivar was also observed at GF (Table 3). Unlike shoot and root mass, the S:R ratio did not show a clear trend among the cultivars, but averaged across the years, it was greater for TAM 304, especially in the wet treatments (Table 4).

### 3.4 Heading date

Heading date was significantly \( (p < .05) \) affected by all main effects, all two-way interactions except year \times cultivar \( (p = .1052) \) and a three-way interaction (Table 1). The three-way interaction was mainly due to the fact that the difference in heading date between TAM 111 and TAM 112 was inconsistent in between dry and wet treatments in both 2012 and 2013 (Figure 1). Overall, in both years, most of the heading occurred within 70 and 75 DAT. Typically, TAM 304 headed about 6–9 days earlier than TAM 111 and TAM 112, and the wet treatment headed about 2-4 days earlier than the dry treatment.

### 3.5 Grain yield

Grain yield was significantly \( (p < .05) \) affected by the main effect of year, water and the year \times cultivar interaction (Table 1). Averaged across the water treatments, there was no cultivar difference for grain yield in 2012, whereas TAM 304 (13.1 g/pot) had higher yield
than TAM 111 (9.2 g/pot) and TAM 112 (9.6 g/pot) in 2013. Although the cultivar \times water interaction was marginal \((p = .1024)\), averaged across the years, in the dry treatment TAM 112 (19.2 g/pot) had 67% and 81% more grain yield than TAM 111 (11.5 g/pot) and TAM 304 (10.6 g/pot), respectively. Many of the tillers did not produce harvestable grains in 2013 especially in the dry treatment, so the grain yield in 2013 (10.6 g/pot) was less than in 2012 (29.0 g/pot).

### 3.6 Water use efficiency

Water use efficiency for grain (WUEg) was significantly \((p < .05)\) affected by all main effects and all two-way interactions (Table 1). There was no cultivar difference in mean WUEg in 2012, but WUEg was higher in TAM 304 (0.68 kg/m³) than in TAM 111 (0.41 kg/m³) and TAM 112 (0.45 kg/m³) in 2013 (Table 2). In the dry treatment, the mean WUEg was significantly higher in TAM 112 (1.32 kg/m³) than in TAM 111 (0.79 kg/m³) and TAM 304 (0.80 kg/m³). In contrast, in the wet treatment, the mean WUEg was significantly higher in TAM 304 (1.09 kg/m³) than in TAM 111 (0.75 kg/m³) and TAM 112 (0.74 kg/m³). This was mainly due to the fact that compared to TAM 111 and TAM 112, TAM 304 produced a numerically greater grain yield in the wet treatment (Table 2).

Water use efficiency for biomass (WUEbm) at maturity was significantly \((p < .05)\) affected by the main effects of year and cultivar, and by all two-way interactions. The mean WUEbm was not

### Table 3

Analysis of variance (p-values) for wheat shoot mass, root mass and shoot-to-root ratio (S:R), at heading (HD), mid-grain filling (GF) and physiological maturity (MT) stages

<table>
<thead>
<tr>
<th>Effect</th>
<th>Shoot mass</th>
<th>Root mass</th>
<th>S:R ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HD</td>
<td>GF</td>
<td>MT</td>
</tr>
<tr>
<td>Year (Y)</td>
<td>0.0798</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Cultivar (C)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.0006</td>
</tr>
<tr>
<td>Water (W)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Y \times C</td>
<td>0.0005</td>
<td>0.0046</td>
<td>0.0434</td>
</tr>
<tr>
<td>Y \times W</td>
<td>&lt;.0001</td>
<td>0.0041</td>
<td>0.2282</td>
</tr>
<tr>
<td>C \times W</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.8733</td>
</tr>
<tr>
<td>Y \times C \times W</td>
<td>0.1057</td>
<td>0.0985</td>
<td>0.7522</td>
</tr>
</tbody>
</table>

### Table 4

Means of wheat shoot mass, root mass and shoot-to-root ratio (S:R), at heading (HD), mid-grain filling (GF) and maturity (MT) stages, as affected by year or water and cultivar interaction

<table>
<thead>
<tr>
<th>Year/water Cultivar</th>
<th>Shoot mass (g/pot)</th>
<th>Root mass (g/pot)</th>
<th>S:R ratio (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HD</td>
<td>GF</td>
<td>MT</td>
</tr>
<tr>
<td>TAM 111 2012</td>
<td>23.7 a</td>
<td>46.8 b</td>
<td>66.5 a</td>
</tr>
<tr>
<td>TAM 112 2012</td>
<td>23.3 a</td>
<td>51.8 a</td>
<td>69.7 a</td>
</tr>
<tr>
<td>TAM 304 2012</td>
<td>15.5 b</td>
<td>37.2 c</td>
<td>60.6 b</td>
</tr>
<tr>
<td>Mean</td>
<td>20.8 A**</td>
<td>45.2 A</td>
<td>65.6 A</td>
</tr>
<tr>
<td>TAM 111 2013</td>
<td>25.4 a</td>
<td>40.7 a</td>
<td>46.0 a</td>
</tr>
<tr>
<td>TAM 112 2013</td>
<td>20.6 b</td>
<td>38.8 a</td>
<td>42.8 ab</td>
</tr>
<tr>
<td>TAM 304 2013</td>
<td>21.0 b</td>
<td>36.7 a</td>
<td>39.6 b</td>
</tr>
<tr>
<td>Mean</td>
<td>22.3 A</td>
<td>38.7 B</td>
<td>42.8 B</td>
</tr>
<tr>
<td>TAM 111 Dry</td>
<td>15.8 a</td>
<td>23.7 a</td>
<td>34.1 a</td>
</tr>
<tr>
<td>TAM 112 Dry</td>
<td>15.1 a</td>
<td>25.4 a</td>
<td>34.7 a</td>
</tr>
<tr>
<td>TAM 304 Dry</td>
<td>14.1 b</td>
<td>24.5 a</td>
<td>29.1 a</td>
</tr>
<tr>
<td>Mean</td>
<td>15.0 B</td>
<td>24.5 B</td>
<td>32.6 B</td>
</tr>
<tr>
<td>TAM 111 Wet</td>
<td>33.1 a</td>
<td>61.8 a</td>
<td>78.4 a</td>
</tr>
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<td>TAM 112 Wet</td>
<td>28.1 b</td>
<td>67.8 a</td>
<td>77.0 a</td>
</tr>
<tr>
<td>TAM 304 Wet</td>
<td>22.3 c</td>
<td>49.1 b</td>
<td>70.8 a</td>
</tr>
<tr>
<td>Mean</td>
<td>27.8 A</td>
<td>59.6 A</td>
<td>75.4 A</td>
</tr>
</tbody>
</table>

*In each column, means with different lowercase letters under each year or water level are significantly different at \(p < .05\) based on LSD test.

**In each column, means with different uppercase letters under year or water are significantly different at \(p < .05\) based on LSD test.
different among the cultivars in both years, but TAM 304 in the dry treatment and TAM 112 in the wet treatment had lower mean WUEbm compared to other cultivars (Table 2).

3.7 | Photosynthetic parameters

Trends in photosynthetic parameters were similar between the two experiments. Overall, leaf chlorophyll content was greater in TAM 111 followed by TAM 112 and TAM 304, which decreased as the growth stage progressed (Figure 2). The SPAD values in both wet and dry treatments in 2013 were lower than in 2012 at the later growth stage. This probably was due to the early leaf senescence associated with low temperature (cold) stress. The gas exchange parameters (Gs, Pn, Ci and Tr) for all cultivars decreased from the wet to dry treatment. The difference in gas exchange among the cultivars was more evident in the dry treatment than in wet, and in 2013 than in 2012 (Figures 3 and 4). The overall reduction in Pn from wet to dry treatment and from morning to afternoon was likely due to the increased water stress resulting in the closure of stomata. In 2012, in the wet treatment, most of the values of the different gas exchange parameters were similar among the cultivars, but there was a clear trend that TAM 112 had lower Gs, Pn, Ci and Tr, but higher PWUE, especially at 11:00 hrs, compared to TAM 111 and TAM 304 (Figure 3a–e). The dry treatment resulted in significantly \( p < .05 \) lower Gs, Pn and Tr, but higher PWUE in TAM 112 than in TAM 111 and TAM 304 at 11:00 hrs, but there was no cultivar difference in these parameters at 16:00 hrs, except lower Ci in TAM 111 and PWUE in TAM 304 (Figure 3f–j).

In 2013, in the wet treatment, TAM 112 had significantly \( p < .05 \) lower Gs and Tr, but higher PWUE in the morning, while there was no cultivar difference in the afternoon (Figure 4a–e). In the dry treatment too, compared to TAM 111 and TAM 304, TAM 112 had significantly \( p < .05 \) lower Gs, Pn, Ci and Tr, but a higher PWUE in the morning, and had a lower Gs and Tr in the afternoon (Figure 4f–j). Between TAM 111 and TAM 304, all the gas exchange parameters at both water treatments and measurement times were similar, except TAM 304 had a significantly \( p < .05 \) lower PWUE in 2012 and Pn in 2013 compared to TAM 111, in the dry treatment, during the afternoon (Figures 3 and 4g). In both years, Pn was positively related to Gs, Tr and Ci (Figure 5). Although the relationships between Pn-Gs and Pn-Tr were curvilinear, Pn decreased as Gs, Tr and Ci decreased.

4 | DISCUSSION

4.1 | Water use, plant growth and yield

In general, biomass production is related to seasonal ET and WUEbm (Blum, 2009; Musick et al., 1994; Passioura, 2007). We found a trend towards a cultivar difference in cumulative ET at both water treatments, but the significant difference was only in the wet treatment. Also, a cultivar difference due to cumulative ET was more evident in 2012 than in 2013 (Table 2). Overall, TAM 111 and TAM 112 had greater cumulative ET than TAM 304 regardless of water treatment and year. As water used for transpiration (T) is intrinsic to the development of plant biomass, cultivars with higher biomass probably had a greater T/ET ratio.

A primary focus in drought tolerance research has been on root morphology and biomass, which may be beneficial to improving yield and yield stability. Averaged across the years and cultivars, compared to the wet, the dry treatment had lower plant height and tiller number which eventually reflected in lower AGB and grain yield. The root mass was also lower in the dry than in the wet treatment. This phenomenon revealed that plant height, number of tillers and root mass were important determinants for wheat yield. In general, AGB was higher for TAM 111 and TAM 112 compared to TAM 304 (Table 4). Under water-limited environments, Xue et al. (2014) also found higher biomass and grain yield in TAM 111 and TAM 112 as compared to other genotypes.

Among the three cultivars used in this study, TAM 112 has 1AL.1RS rye translocations. Under some genetic backgrounds, the 1RS translocation can be more adapted to drought conditions and increase yield due to the increased root biomass (Echdaie, Whitkus, & Waines, 2003; Villareal, Banuelos, Mujeeb-Kazi, & Rajaram, 1998; Xue et al., 2014). This was found true in the dry treatment, where TAM 112 produced greater grain yield than TAM 111 and TAM 304. Averaged across the water treatments, there was no cultivar difference in yield in 2012, but TAM 304 produced significantly...
higher grain yield than TAM 111 and TAM 112 in 2013. The yield difference in 2013 was mainly due to the cold stress (about a week) during the time of heading to flowering, due to the glasshouse heaters failing. As TAM 304 produced heads about 6–9 days earlier than TAM 111 and TAM 112 (Figure 1), it may have avoided the cold stress. Wheat is most sensitive to freeze injury during the reproductive period, which begins with pollination during late booting or heading stage (Klein, 1994). The low temperature at heading/flowering stage probably resulted in floral abnormalities, low pollen viability and pollination inhibition resulting in grain yield loss. Reduced AGB as well as grain yield in 2013 was also due to more severe water stress in the dry treatment, in which soil water was maintained at 40% pot capacity, compared to 50% pot capacity in 2012.

At maturity, averaged across the years, compared to TAM 111 and TAM 112, the S:R ratio was consistently greater for TAM 304 in the wet treatment, while the dry treatment showed mixed results (Table 4). This result indicated that TAM 304 was more suitable for an adequate water supply than limited, which was also suggested by Rudd et al. (2015). Further, in the wet treatment, TAM 304 had higher WUEg than TAM 111 and TAM 112, indicating that TAM 304 was able to utilize water more efficiently in the wet treatment. Similar to WUEg, TAM 304 had equal or greater WUEbm in the wet treatment, but lower WUEbm in the dry treatment. In contrast, in the dry treatment, TAM 112 had higher WUEg and WUEbm, suggesting that TAM 112 was more suitable for a water-limited condition compared to TAM 111 and TAM 304.

4.2 | Photosynthetic parameters

Physiological parameters for evaluating water-deficit response in crops include leaf water potential, chlorophyll content, Pn, Gs and Tr (Araus, Slaf er, Royo, & Serret, 2008). Our results on leaf chlorophyll content indicated that leaf senescence in TAM 304 was started earlier than in TAM 111 and TAM 112, which could be partially due to the early heading in TAM 304. Overall, at different dates, the chlorophyll content was greater in TAM 111 followed by TAM 112 and TAM 304 (Figure 2). According to Anjum, Xie, L-c Wang, Saleem, and Lei (2011), chlorophyll content can be used as a measure of dry-matter accumulation status under water stress. Paknejad, Nasri, Moghadam, Zahedi, and Alahmadi (2007) reported a positive correlation between chlorophyll content and grain yield in bread wheat cultivars under drought. Abdipur, Ramezani, Bavei, and Talaei (2013) conducted a study in bread wheat and used chlorophyll content as a reliable indicator for evaluating the integrity of the photosynthetic apparatus under stress. They further used chlorophyll content as a selective tool for higher grain yield potential under a water-deficit environment. In our study, TAM 111 and TAM 112 had more chlorophyll than TAM 304 in the dry treatment, and a correspondingly greater AGB and grain yield. Reduction in chlorophyll content in TAM 304, mainly due to drought stress and early senescence, indicated low concentrations of photosynthetic pigments in TAM 304, which could cause inactivation of photosynthesis and inhibition of photosynthetic potential (Anjum et al., 2011; Loutfy et al., 2012).

The investigation of photosynthetic activity under drought conditions could be enhanced by evaluating gas exchange measurements such as Gs (Flexas, Bota, Loreto, Cornic, & Sharkey, 2004; Long, Ainsworth, Rogers, & Ort, 2004). An increase in water stress decreases turgor pressure inside the cells. Stomata generally respond to this situation by partial closing (Eamus, Taylor, Macinnes-Ng, Shanahan, & De-Silva, 2008; Farquhar, Schulze, & Kfippers, 1980; Lange, Losch, Schulze, & Kappen, 1971) to limit transpiration and eventually conserve water from excessive loss (Kholova et al., 2010; Sinclair, Hammer, & Van Oosterom, 2005). As reported by Oren et al. (1999), such phenomena may frequently occur around midday,
which prevents extreme dehydration to the plants. As such, TAM 112 probably closed stomata either partially or fully to respond to water stress, so Gs, Pn, CI and Tr were generally reduced both in dry and wet treatments and in 2012 and 2013 (Figures 3 and 4).

The interesting consequence of this response was the remarkable increase in PWUE in TAM 112 than in TAM 111 and TAM 304, indicating that TAM 1112 was able to tolerate drought stress more effectively. Further, gas exchange parameters generally were similar

FIGURE 3  Physiological parameters for different cultivars at different water treatments measured during the morning (11:00 hrs) and afternoon (16:00 hrs) at mid-grain filling stage in 2012. The vertical line above the bars denotes standard error of the mean, and bars with different letters on the top are significantly different at \( p < .05 \). Gs = stomatal conductance; Pn = net photosynthetic rate; CI = intercellular CO\(_2\) concentration; Tr = transpiration rate; PWUE = photosynthetic water use efficiency
between TAM 111 and TAM 304, but TAM 111 had greater chlorophyll and PWUE in the dry treatment. Consequently, in the dry treatment, aboveground biomass at maturity and grain yield were numerically greater in TAM 112, followed by TAM 111 and TAM 304 (Tables 2 and 4).

Compared to wet, wheat plants in the dry treatment generally had lower Gs, Tr and Ci, leading to reduced Pn. Similar results have been reported in other studies (Ghaderi, Talaie, Ebadi, & Lessani, 2011; Saeidi & Abdoli, 2015; Siddique, Hamid, & Islam, 2000; Stiller, Read, Constable, & Reid, 2005). In this study, despite lower Gs, Pn, Ci and Tr, TAM 112 had higher PWUE and produced higher grain yield than TAM 111 and TAM 304 in the dry treatment. Hence, it is likely that the relationship among Gs, Pn, water stress and crop yield is complex, which may depend on various conditions, such as the rate and severity of the water stress, crop species, the age of plant and the amount of solar radiation.

FIGURE 4 Physiological parameters for different cultivars at different water treatments measured during the morning (11:00 hrs) and afternoon (16:00 hrs) at mid-grain filling stage in 2013. The vertical line above the bars denotes standard error of the mean, and bars with different letters on the top are significantly different at $p < .05$. Gs = stomatal conductance; Pn = net photosynthetic rate; Ci = intercellular CO$_2$ concentration; Tr = transpiration rate; PWUE = photosynthetic water use efficiency.
5 | CONCLUSIONS

Morphological traits such as plant height, tiller number and root mass contributed to the aboveground biomass and grain yield suggesting the possibility of evaluating yield performance of cultivars using these attributes, especially in water-limited conditions. Overall, TAM 112 had lower stomatal conductance and other gas exchange parameters, but higher photosynthetic water use efficiency during the grain filling stage, especially in the dry treatment in both years. This feature probably helped TAM 112 to adapt more effectively to the water-stressed condition. Consequently, compared to TAM 111 and TAM 304, TAM 112 produced more grain yield and had similar or greater WUEg and WUEbm in the dry treatment. Differences among the cultivars in terms of gas exchange parameters and yield invite further investigation into leaf anatomy and physiology to better evaluate drought tolerance among these wheat genotypes under field conditions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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