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Physiology and transcriptomics of water-deficit stress responses in wheat cultivars TAM 111 and TAM 112

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SUMMARY

Hard red winter wheat crops on the U.S. Southern Great Plains often experience moderate to severe drought stress, especially during the grain filling stage, resulting in significant yield losses. Cultivars TAM 111 and TAM 112 are widely cultivated in the region, share parentage and showed superior but distinct adaption mechanisms under water-deficit (WD) conditions. Nevertheless, the physiological and molecular basis of their adaptation remains unknown. A greenhouse study was conducted to understand the differences in the physiological and transcriptomic responses of TAM 111 and TAM 112 to WD stress. Whole-plant data indicated that TAM 112 used more water, produced more biomass and grain yield under WD compared to TAM 111. Leaf-level data at the grain filling stage indicated that TAM 112 had elevated abscisic acid (ABA) content and reduced stomatal conductance and photosynthesis as compared to TAM 111. Sustained WD during the grain filling stage also resulted in greater flag leaf transcriptome changes in TAM 112 than TAM 111. Transcripts associated with photosynthesis, carbohydrate metabolism, phytohormone metabolism, and other dehydration responses were uniquely regulated between cultivars. These results suggested a differential role for ABA in regulating physiological and transcriptomic changes associated with WD stress and potential involvement in the superior adaptation and yield of TAM 112.

Introduction

Wheat is the second most important cereal crop in the United States and winter wheat comprises 70% of the cultivated wheat area (http://www.nass.usda.gov/). The Southern Great Plains (SGP) of the United States produces over half of the total US winter wheat crop but is often plagued by severe to extreme drought. Breeding for drought tolerance under such low-yielding environments with high evaporative demand is a complex process. When breeding for drought tolerance and sustained yield gains, it is necessary to understand the physiological and molecular basis of stress tolerance and employ an integrated approach (Araus et al., 2002; Blum, 2011; Reynolds et al., 2012).

Plant performance and yield responses to water-deficit (WD) stress conditions have been extensively studied (Blum, 2009; Chaves et al., 2003; Lawlor, 2013). Crops may employ a variety of strategies for successful production under water-limiting environments such as resistance, tolerance, avoidance, survival, and escape, or combinations thereof (Chaves et al., 2003; Passioura, 2007). Phenotypic adaptation to WD stress at the whole-plant level includes alterations in canopy architecture, root-to-shoot ratio, plant height, above ground biomass, tiller and spike number, grain number, and grain weight. The terminal phenotypic traits are mainly driven by photosynthesis and associated carbon allocation and partitioning, and competition between the sink tissues. WD conditions have been shown to limit photosynthetic rate and affect yield; however, the causal mechanisms are complex owing to

Abbreviations: *A*, photosynthesis; ABA, abscisic acid; BR, brassinosteroid; C_i/C_a, ratio of intercellular CO_2 to ambient CO_2; CK, cytokinin; *E*, transpiration; ET, ethylene; FC, fold change; GA, gibberellin; g_s, stomatal conductance; SGP, Southern Great Plains; WD, water deficit.

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inherent differences in photosynthesis, efficiency, and interactions with the dynamic environment (Lawlor and Tezara, 2009).

WD stress responses at the cellular level induce osmotic adjustment, reduced transpiration, varied CO_2 diffusion, and decreased photosynthesis due to stomatal closure (Lawlor and Tezara, 2009). WD induced changes at the molecular level are mediated by numerous signals. The key responses and core signaling components include photosynthetic byproducts such as sugars and reactive oxygen species (ROS), phytohormones, transcription factors, amino acids, and other metabolic signals (Gong et al., 2010; Lawlor, 2013; Pinheiro and Chaves, 2011). The phytohormone abscisic acid (ABA) has been proposed to regulate a reduction in photosynthesis and mediate photosynthate remobilization between source and sink tissues, while other hormones also play key roles (Pinheiro and Chaves, 2011; Wilkinson et al., 2012).

Transcriptomic, proteomic, and metabolomic studies have increased the understanding of WD stress responses at the molecular level (Harb et al., 2010; Padmalatha et al., 2012; Payton et al., 2009; Seiler et al., 2011). Gene expression profiling of wheat subjected to biotic and abiotic stress has been conducted previously (Aprile et al., 2009; Ergen et al., 2009; Krugman et al., 2010; Mohammadi et al., 2007; Reddy et al., 2013b; Szucs et al., 2010; Xue et al., 2006). However, these studies were not conducted in widely planted commercial wheat cultivars with distinct adaptation mechanisms. The results of "omics" studies targeting commercial varieties may support breeding efforts to increase grain yield and sustain productivity (Langridge and Fleury, 2011; Tester and Langridge, 2010).

The hard red winter wheat cultivars TAM 111 and TAM 112 (Lazar et al., 2004; Rudd et al., 2014), are widely cultivated on the SGP and continue to gain popularity among producers. The germplasm resources of these cultivars are used by breeding programs in the US and around the world to improve drought tolerance in arid and semi-arid production regions. TAM 112 had high yield stability and performed better in most environments including low yield potential environments, while TAM 111 is superior in high input environments (Battenfield et al., 2013). Studies also suggested the cultivars show altered growth responses to variable WD conditions and potentially distinct adaptation mechanisms (Xue et al., 2014). However, the physiological and molecular basis of their drought tolerance mechanisms remains unknown. The wide adaptability, distinct drought tolerance responses, and their popularity among scientific and farming communities make TAM 111 and TAM 112 ideal cultivars for studying important traits like drought tolerance.

We hypothesized that the distinct stress adaptation observed in the TAM cultivars is a resultant of altered responses at gene and whole-plant level, and can be explained using relevant transcriptomic and physiological studies. The results suggested a key role for the phytohormone ABA in altering gene expression and photosynthetic parameters and generated several hypotheses for further investigation.

Materials and methods

Plant materials, growth conditions, and treatments

Hard-red winter wheat cultivars TAM 111 and TAM 112 were used. The cultivars partially share pedigree and were developed by Texas A&M AgriLife Research. The pedigree for TAM 111 (TAM 107//TX78V3620/CTK78/3/TX87V1233) includes TAM 107, Centurk, and Texas experimental lines, while TAM 112 (TX98V9628 = U1254-7-9-2-1/TXGH10440) is derived from TAM 110 sib, TAM 200, and Kansas experimental lines. Greenhouse experiments were conducted at the Texas A&M AgriLife Research and Extension Center at Bushland, Texas USA (35°11′ 31″ N/102°3′53″ W). Seeds were planted in LC1 potting mixture (Sun Gro Horticulture Canada Ltd.). After emergence, the seedlings were vernalized for 7 weeks at 4 °C in a growth chamber. Seedlings were then transplanted into two gallon pots filled with Calcined Fullers Earth (PrimeraOne®, OH, USA) and moved into a greenhouse. Osmocote Controlled Release fertilizer (19-6-12; 100 ppm N) was thoroughly mixed in the soil and another 100 ppm N of Miracle-Gro (24-8-16) plant food was added in four intervals along with irrigation water during early seedling establishment. To emulate natural conditions, greenhouse temperatures were periodically adjusted. The day/night temperatures were initially set to 18/10 °C. At the start of the WD stress treatment the temperatures were increased to 22/14 °C, and increased further to 26/18 °C during grain filling.

Five seedlings were transplanted into each pot and rubber mulch was added to minimize evaporation. At 14 days after transplanting (DAT), the pots were thinned to retain three healthy seedlings. Prior to the WD stress treatments, the pots were maintained at 100% gravimetric water content (GWC) for 7 weeks. After WD treatments started (50 DAT; jointing stage), during each irrigation event (three times a week) the control pots were brought back to 90% GWC and the WD treatment were brought back to 50% GWC. A confounded factorial design was used in the study with three replicates for each cultivar, irrigation treatment (wet and dry), and sampling stage (heading, grain filling, and physiological maturity). Sampling stages were treated as blocks within the replication and each block included a randomized arrangement of cultivar and irrigation treatments.

Gas exchange, chlorophyll, and other physiological measurements

Measurements of net photosynthesis (A), stomatal conductance (g_s), transpiration (E), and the ratio of intercellular to ambient [CO_2] (C_i/C_a) were made on flag leaves of primary shoots using a Licor 6400XT (Licor, Lincoln, NE, USA). Key physiological and molecular measurements were made on flag leaves to develop methods for trait characterization, given the role of flag leaves in determining grain yield in wheat (Guoth et al., 2009; Hui et al., 2008). All measurements were made at saturating light levels (1800 $\mu mol\ m^{-2}\ s^{-1}$), ambient growth [CO_2] (385 $\mu l\ l^{-1}$), mid-day growth temperature (29 °C), and a leaf vapor pressure deficit ranging from 0.7 to 2.4. Measurements were made twice a day at 3 (AM) and 8 (PM) h after the start of the photoperiod. Three and six plants were measured for each genotype and treatment during the 2012 and 2013 studies respectively at the grain filling stage when the GWC of most pots was between 70–80% and 40–50% for wet and dry treatments, respectively. Trends in photosynthetic parameters were similar across the years. Results from 2013 are presented and discussed in the manuscript, while data from 2012 can be accessed in Supplementary Fig. S1.

Chlorophyll content of flag leaves of the primary shoots was measured using a Konica Minolta SPAD 502 meter. Cumulative transpiration, tiller number, plant height, above ground biomass (AGB), and below ground biomass (BGB) were measured at different developmental stages. Stems, leaves, roots, and spikes were harvested separately at physiological maturity to determine the grain yield and other yield parameters.

Tissue sampling, RNA extraction, and microarray assay

Transcriptome profiling was conducted on flag leaves at heading (79 DAT) and grain filling stages (100 DAT). The flag leaves of primary shoots from four individual plants were harvested together and pooled to make one sample. Samples were harvested into liquid N_2 and stored at −80 °C until processing.