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Transpiration Ratio in Sorghum [*Sorghum bicolor* (L.) Moench] for Increased Water-use Efficiency and Drought Tolerance

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Abstract: There is an increasing need to improve crop water-use efficiency (WUE) (i.e., the ratio of whole-plant biomass to cumulative transpiration) due to decreased water availability and increased food and energy demands throughout the world. In previous studies, the pre-flowering transpiration ratio (A:E) [CO_2 assimilation rate (A) divided by transpiration rate (E)] of sorghum leaves was correlated with WUE. The present greenhouse study was conducted to examine pre-flower A:E in 70 inbred sorghum (*Sorghum bicolor* L. Moench) lines and two parents (Tx430 and Tx7078) in terms of phenotypic and genetic variation. Parents were selected with contrasting A:E based on previous studies. The experimental design was a Randomized Complete Block, with genotype (70 inbred lines and the parents) and water regime (40 and 80% of "field capacity" (FC)) as experimental factors, and four replications. Genotype had a highly significant effect on A, E and A:E. Frequency distribution for A:E revealed the normal distribution indicating the polygenic segregation among the progenies. Average A:E was 3.07 mmol CO_2 mol^{-1} H_2O for Tx430 and 2.80 for Tx7078. These results provide further evidence that there is genetic variability among genotypes for gas exchange rates at pre-flowering in sorghum suggesting scope for improved WUE and productivity.

Key Words: Drought, Sorghum, Transpiration ratio, WUE

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop after wheat, maize, rice and barley (FAO, 2009) and it is a staple food crop for 500 to 700 million people in arid and semiarid regions primarily of Africa and some of Asia. In terms of utilization, worldwide, almost half of the grain sorghum produced is used as animal feed. More recently sorghum has been proposed as a dedicated cellulosic bioenergy feedstock (Rooney *et al*., 2007).

With the rapid increase in population and scarcity of the fresh water resource, water shortage (or drought) has become the key factor that constrains crop production worldwide. Therefore, strategies to increase the food productivity while conserving the water become increasingly important (Unger and Howell, 1999). These include selection of drought tolerant crops and improvement of WUE. Sorghum is known for its extensive phenotypic and genotypic variation in response to drought (Blum, 1979; Doggett, 1988). The superior drought tolerance in the crop is likely due to its evolution in Sub-Saharan Africa, a region characterized by predictably low and erratic rainfall patterns. Thus, it is one of the most drought tolerant crops and serves as a model for studying the genetic and physiological mechanisms of drought tolerance in cereal crop species. Even though considerable work has been done on plant response to moisture stress (Tuinstra *et al*., 1996; Xu *et al*., 2000; Kebede *et al*., 2001; Haussmann *et al*., 2002), there has been little emphasis on the use of specific physiological traits to enhance the drought stress tolerance. Specific physiological trait like transpiration ratio (A:E), which is defined as change in CO_2 assimilation rate (A) per unit change in transpiration rate (E), is one potential way to increase water productivity under drought stress condition.

1.1. Gas exchange rates and WUE

Several field and greenhouse studies have found significant genetic variation for A:E in grain sorghum under water limited conditions (Krieg and Hutmacher, 1986; Kidambi *et al*., 1990a, Peng *et al*., 1991; Peng and Krieg, 1992; Krieg *et al*., 1992; Balota *et al*., 2008). Significant variation in the ratio of A to stomatal conductance (g) has been observed (Kidambi *et al.* (1990a). In their study, g was relatively conservative, suggesting that it may be possible to select for increased A without a concurrent increase in g in sorghum. Genotypic variation in A was associated with increased leaf area and shoot biomass production; this occurred without significant increase in water use or leaf transpiration (Peng and Krieg, 1992). Further, they concluded that measurements of A and leaf area could be used as selection criteria for higher WUE in grain sorghum under field conditions. Peng *et al*. (1991) reported a strong correlation between A and total biomass production in

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22 sorghum genotypes.

They also suggested that single leaf measurement of A could be used to select for higher productivity among sorghum genotypes. Balota *et al*. (2008) examined four sorghum parental inbred lines and 12 of their hybrids for transpiration ratio under water limited and well watered conditions. They found that average A:E over both water conditions was 3.10 mmol CO_2 mol^{-1} H_2O for Tx430 and 2.91 for Tx7078. These two genotypes also had the highest A. They concluded that there is genetic variation for pre-flower A, E, and A:E rating as well as WUE in sorghum genotypes. Even though pre-flowering drought-stress commonly occurs in sorghum production environments (Rosenow *et al*., 1996), very few genetic analyses have been completed for pre-flowering drought-stress. Therefore, the objective of this research was to determine the genetic variation for A:E related to pre-flower drought tolerance in sorghum recombinant inbred lines population under controlled conditions.

2. Material and Methods

A total of 70 F6 generation recombinant inbred lines (RILs) and two parents (Tx430 and Tx7078) were developed in the Texas AgriLife Breeding Program directed by Dr. William L. Rooney. The parents were selected based on contrasting values of transpiration ratio (A:E). The RILs and their parents were grown in a greenhouse at the Texas AgriLife Research and Extension Station in Bushland, TX, (35°11' N lat; 102°06' W long; 1170 m elevation) to measure gas exchange rates. The experimental design was a randomized complete block with four replications, with genotype and water regime (40 and 80 % FC) as experimental factors.

Each pot contained 2.5 kg finely screened Pullman clay loam soil (fine, mixed, superactive, thermic Torrertic Paleustolls. The two water treatments, i.e. 80 % and 40% FC, were based on soil water retention curves determined by a pressure plate apparatus (Klute, 1986) and correspond to gravimetric soil water contents of 0.17 kg kg^{-1} at a pressure potential of -0.1 MPa and 0.09 kg kg^{-1} at -1 MPa. Each pot was lined with a plastic bag, which was wrapped around the stems to minimize water evaporation from the soil surface. For the first 15 days after planting (DAP) all the pots were maintained under well watered conditions. Water treatments were imposed at 16 d after planting and maintained by daily weighing and watering, using an electronic balance with increments of 5 g. Gas-exchange measurements were taken from 32 - 34 DAP using LI-6400 Infrared Gas Analyzer (IRGA) portable photosynthesis system (LI-COR, Lincoln, NE) on 70 inbred lines and two parents. Genotype effect on A, E, and A:E was analyzed with ANOVA from the GLM procedure of SYSTAT 10.2 (2002, SYSTAT Software Inc., Richmond, CA) using genotype and replication as independent variables and each pot as an experimental unit (Balota *et al*., 2008).

3. Results and Discussion

The RILs for mean values of A:E, A and E were normal distributed, as expected for a quantitative trait both under 80% and 40% FC except in A:E under 40% FC (**Fig. 1**). The normal distribution of these traits indicated polygenic segregation. However, skewed distribution of A:E under 40% FC suggested the involvement of a single gene with large effects controlling this physiological trait.

Genotype had a highly significant effect on A ($P < 0.0001$ and $P<0.0001$), E ($P < 0.002$ and $P<0.007$) and A:E ($P < 0.0001$ and $P<0.0001$) under 80% and 40% FC, respectively (**Table 1**). Genetic variation in A and E among sorghum lines and their hybrids have been reported previously by several authors (Kidambi *et al*., 1990b: Peng and Krieg, 1992; Balota *et al*., 2008). Kidambi *et al*. (1990a) reported substantial genetic variation in the A: g (stomatal conductance) relationship that caused by significant genetic variation in A. However, g was relatively more conservative to increasing water stress. They proposed that selection for high A might directly contribute to greater WUE and higher drought tolerance. However, Balota *et al*. (2008) suggested that concomitant selection for high A:E and A may be necessary when high biomass and WUE are both desired. In our study, we found genotypes had highly significant effect on A, E and A:E, which provides further evidence that when we desired to have potentially greater drought tolerance, it might be necessary to select for both higher A:E and A.

Between the two levels of water regimes in experiment 1, values for A, E, and A:E were generally larger under 80% FC than those in the 40% FC among the RILs (**Table 2**). However, RILs produced ~10% more biomass per unit of transpiration under 40% FC than 80% FC in experiment 1. In the study by Balota *et al*. (2008), which included both low water (LW) and high water treatment, LW treatment resulted in lower biomass and cumulative transpiration in sorghum similar to our study. They predicted that lower biomass under LW might be due to the acclimatization of plants to LW by regulating the transpiration without partial stomatal closure which is more sensitive to moisture stress.

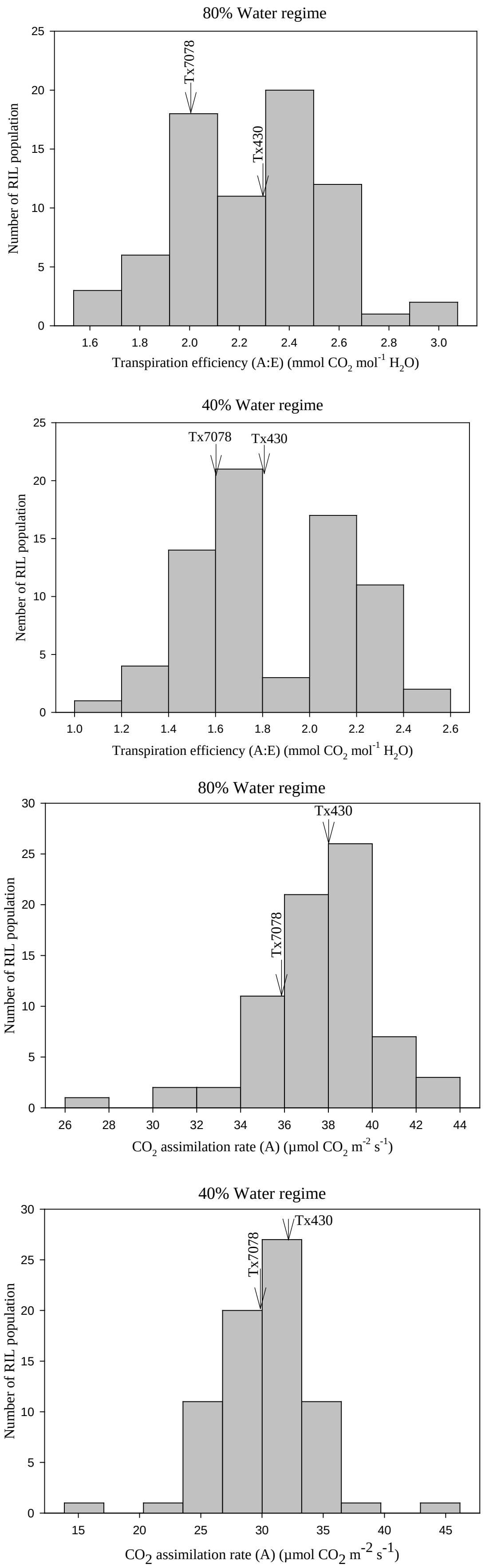

Fig. 1. Frequency distribution of transpiration ratio (A:E) and CO_2 assimilation rate (A) at 80% and 40% FC in 70 recombinant inbred lines and two parents. The mean A:E values for Tx7078 and Tx430 are indicated by arrows.

Table 1. Mean squares and probability levels (*p*) by environment (water regime) from ANOVAs for gas exchange traits related to pre-flower drought tolerance in sorghum RIL population derived from Tx430 x Tx7078, Bushland, Texas during 2008.

Source	Df	A		A:E	
		MS¶	*p*	MS	*p*
Genotype	72	70	<0.001	0.88	<0.001
Replication	3	823	<0.001	132	<0.001
Error	681	38		0.19	
Genotype	72	114	<0.001	1.02	<0.001
Replication	3	4146	<0.001	116	<0.001
Error	601	32		0.09	

Units: A- µmol CO_2 m^{-2} s^{-1}; A:E - mmol CO_2 mol^{-1} H_2O kPa^{-1}; ¶Mean square.

Table 2. Mean values for CO_2 assimilation rate (A), transpiration (E) and transpiration ratio (A:E) for recombinant inbred lines (RILs), Bushland, TX.

Trait	RILs mean (SD)†	
	40% FC	80%FC
CO_2 assimilation rate (A)	30.12	37.21
(µmol CO_2 m^{-2} s^{-1})	(4.14)†	-2.68
Transpiration (E)	6.65	8.37
(mmol H_2O m^{-2} s^{-1})	-0.73	-0.72
Transpiration ratio (A:E)	1.93	2.29
(mmol CO_2 mol^{-1} H_2O)	-0.44	-0.31

†Standard deviation

4. Conclusion

These results provide further evidence that there is genetic variability among genotypes for gas exchange rates (A, E and A:E) at pre-flowering in sorghum suggesting scope for improved WUE and productivity. We believe that our results are potentially useful to develop the genetic map and identify the genes involved in pre-flower drought tolerance, particularly at the GS2 stage, and increased sorghum production under U.S. Great Plains environments, Africa and parts of India.

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