



Genetic diversity of transpiration efficiency in sorghum[☆]

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ABSTRACT

Sorghum is the fifth most important grain crop and is becoming increasingly important as a biofuel feedstock due to its superior tolerance to water-deficit stress. Sorghum is commonly grown under rain-fed conditions in the Southern Plains in the U.S.A. and other semi-arid regions in the world. Thus, its production is strongly affected by the availability of soil water during the growing season. Enhancing transpiration efficiency (TE), defined as biomass accumulation per unit water transpired, may be an effective approach to increasing sorghum yield in arid and semi-arid regions under no or limited irrigation. In this report, we surveyed 341 sorghum accessions for variation in TE. A selection of 25 lines was used to confirm the initial survey and these accessions were studied in a greenhouse experiment over 2 years. TE in these selected lines varied from year to year; however, similar rank was observed in both studies. Several accessions with consistent high or low TE were identified. TE based on biomass production was strongly correlated with increased biomass accumulation rather than with reduced water use. Gas-exchange analysis indicated that low internal CO₂ concentration and enhanced photosynthetic capacity may be a factor accounting for the high TE in some lines; other physiological processes also contribute to the TE based on integrated biomass. The result suggests that considerable genetic variation in TE exists in the sorghum germplasm collection and that TE is strongly influenced by environment. The sorghum lines with contrasting TE may serve as an important genetic resource for identification of physiological mechanisms regulating TE and for improvement of this trait in sorghum breeding.

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1. Introduction

Worldwide, agriculture consumes over 70% of fresh water resources used annually (Bacon, 2004). The rapid decline in fresh water resources, coupled with the demand for increased food production to meet the population growth, poses a great challenge to agriculture. To maintain or further increase agriculture output depends in part on the efficient management of water to maximize water productivity, the concept of assessing agricultural output based on water consumed rather than land area (Bennett, 2003;

Hamdy et al., 2003). Improvement in irrigation technology and agricultural practices that reduce water losses through soil surface evaporation or run-off have played a significant role in increased water productivity (Howell, 2001). Improvement of transpiration efficiency (TE) of crops, the inherent water use efficiency, defined as biomass produced per unit water transpired through plants, may be another viable approach to increase water productivity (Condon et al., 2004).

As a C₄ crop, sorghum [*Sorghum bicolor* (L.) Moench] possesses high TE and is well adapted to semi-arid environments (Doggett, 1988; Rooney, 2004). It is the fifth most important grain crop worldwide and provides staple food for millions of people in the semi-arid tropics of Africa and Asia (www.fao.org). Recently, sorghum has become the second-ranked feedstock for grain-based ethanol in the US after maize. Sorghum is particularly advantageous, as it can be grown on marginal land without competing against fiber crops and other food crops for production on fertile land. Due to its superior drought tolerance, sorghum is often grown on land with little or no irrigation on the Great Plains of the US and many other areas in the world. Thus, sorghum yield is contingent on the available water from rainfall and soil water preserved from

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Abbreviations: A, leaf assimilation rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); C_i, carbon dioxide concentration in sub-stomatal cavity ($\mu\text{mol mol}^{-1}$); Δ , carbon isotope ¹³C/¹²C discrimination ratio; g_s, stomatal conductance to water vapor ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$); nTE, normalized transpiration efficiency ($\mu\text{mol CO}_2 \text{ kPa mmol}^{-1} \text{ H}_2\text{O}$); PEP, phosphoenol pyruvate; T, leaf transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$); TE, transpiration efficiency; VPD, vapor pressure deficit; WUE, water use efficiency.

the prior season. Limitations on maximum transpiration rates, expected to coincide with elevated vapor pressure deficit (VPD), could increase sorghum grain yield by 9–13% in water-deficit environments, e.g., where expected grain yields are less than 4.5 Mg ha⁻¹, according to a simulation study (Sinclair et al., 2005). Improvement in TE is likely to have a large impact on sorghum yield under water-limited conditions.

Although several physiological parameters are correlated with increased TE in sorghum, it is not clear which physiological trait, or combination of traits, is critical to TE (Mortlock and Hammer, 1999). Physiological mechanisms altering TE may involve stomatal regulation of gas-exchange. TE, adjusted for VPD, can increase with declining partial pressure of CO₂ within leaf intercellular spaces (Farquhar et al., 1982; Tanner and Sinclair, 1983). Masle et al. showed that TE can be increased in *Arabidopsis thaliana* by reducing the expression of the gene *ERECTA*, which reduced stomatal frequency (Masle et al., 2005). Leakage of CO₂ from bundle sheath cells can reduce TE because additional reducing power is required to regenerate phosphoenol pyruvate (PEP) that is consumed in the subsequent repeated CO₂ fixation by PEP carboxylase.

Differences in TE among species are well known (Tanner and Sinclair, 1983). In general, plants with the C₄ photosynthesis pathway are more efficient in water use than plants with the C₃ photosynthesis pathway (Bacon, 2004). However, variation in TE within a species has been a subject of debate. Tanner and Sinclair reviewed historic studies on TE, concluding that TE for total biomass is a relatively constant trait within a species (Tanner and Sinclair, 1983). Farquhar et al. established that carbon isotope discrimination ratio $\Delta^{13}\text{C}$ is correlated with TE in C₃ plants (Farquhar et al., 1982). Subsequently, variation in TE within species was demonstrated for several C₃ plant species including wheat (Farquhar and Richards, 1984; Merah et al., 2001; Rebetzke et al., 2002), barley (Teulat et al., 2002), rice (Impa et al., 2005), cotton (Stiller et al., 2005), beans (Ehleringer, 1990), tomato (Martin et al., 1999), sunflower (Lambrides et al., 2004), *Arabidopsis* (Masle et al., 2005), and other C₃ plants. TE variation within species has also been identified in C₄ plants using gas-exchange analysis and gravimetric assays (Peng and Krieg, 1992; Hammer et al., 1997) since the method of carbon isotope discrimination ratio is not easily applicable to C₄ plants (Farquhar, 1983). Genetic variation in TE has also been found in sorghum using gas-exchange properties, traditional lysimetric assays, and field evaluation (Kidambi et al., 1990; Donatelli et al., 1992; Peng and Krieg, 1992; Hammer et al., 1997; Henderson et al., 1998; Mortlock and Hammer, 1999).

Due to the complexity of the assays for TE, only a limited number of lines were examined in these studies. To study the physiological components regulating TE, a series of sorghum lines that have contrasting TE is needed. The objectives of this research were to identify sorghum lines differing in TE from a core collection of sorghum germplasm and to evaluate physiological factors contributing to the differences in TE among these lines. Here, we report the screening and identification of sorghum lines differing in TE.

2. Materials and methods

2.1. Selection of sorghum accessions

To identify the sorghum lines that can be used as key tools to study the mechanism of TE, we assembled a core collection of sorghum lines including 318 Plant Introduction (PI) accessions from more than 3000 photoperiod-insensitive accessions in the world collection at the National Germplasm Resources of USDA-ARS (<http://www.ars-grin.gov/>). This collection was selected to

represent all working groups and geographic regions in the world where sorghum is grown. In addition, the collection includes 23 publicly available sorghum inbred lines. A total of 341 accessions (Supplement Table S1) were surveyed for TE during vegetative growth stage under greenhouse conditions.

2.2. Determination of transpiration efficiency

TE was determined by a high throughput gravimetric method (Xin et al., 2008). Briefly, 2-l plastic pots (14 cm diameter and 16 cm height) were filled with Sunshine #1 potting mix (Sun Gro Horticulture Inc., Bellevue, WA) and watered with 0.5X Miracle-Gro (Scotts Miracle-Gro Products, Inc., Marysville, OH) until dripping from the bottom. Three seeds were planted per pot. After planting, the pots were covered with a layer of dry potting mix to reduce water loss from the soil surface. One week after emergence, each pot was thinned to one plant, and the pot was covered from both ends with 2 Mil poly bags (S-3478, Uline, Waukegan, IL), which are permeable to air but impermeable to water vapor (Xin et al., 2008). A slit was cut in the top bag to permit seedling growth. The slit was further sealed with a piece of clear adhesive tape and covered with a layer of dry potting mix to minimize water loss through the slit. The pots were bar-coded, and the initial weight recorded. The final pot weight was recorded at the time when the plant was harvested. Water used ($W_{\text{transpiration}}$) was calculated by subtracting the final pot weight from the initial weight. Roots were collected by washing the potting mix core on a mesh. Dry weight of roots (M_{root}) and shoots (M_{shoot}) were measured after a minimum of 72 h drying at 80 °C. Total biomass (M_{total}) was calculated as the sum of root and shoot mass. TE was calculated on a shoot basis (TE_{shoot}) as the ratio (M_{shoot}) to ($W_{\text{transpiration}}$), and on a total dry weight basis (TE_{total}) as the ratio (M_{total}) to ($W_{\text{transpiration}}$).

2.3. Survey of transpiration efficiency in core sorghum accessions

The TE protocol, described above, was applied to survey TE in the core collection of sorghum lines. In this survey, two pots were used for each accession. In one pot, the plant was harvested when the seventh leaf ligule became visible—generally, when no obvious stress was observed. In the second pot, the plant was harvested after permanent wilt, defined by the inability of the youngest fully extended leaf (usually the eighth leaf) to regain turgor in the morning. The 341 accessions were evaluated in 29 batches from November 2, 2004 to May 26, 2006. Each batch was completed in approximately 1 month. In each batch, Liang Tang Ai and Tx7078, previously identified as a high and a low TE line, respectively, were included as controls (Xin et al., 2008). In each survey, the TE values were separated into five classes from highest to lowest TE. The relative TE for each accession to Liang Tang Ai, which was set as 100%, was used to compare TE lines from different batches of the surveys. Relative TE was calculated from the average of the two pots, and divided by the corresponding average TE of the Liang Tang Ai for that batch. Relative TE was calculated on both shoot ($\text{RTE}_{\text{shoot}}$) and total ($\text{RTE}_{\text{total}}$) biomass basis. Each survey took from 1 month to 42 days to complete depending on the temperature and VPD during the survey period. All lines remained at vegetative growth stage during the survey.

2.4. Confirmation of the variation in transpiration efficiency

Sixteen PI accessions, representing TE extremes from the initial surveys, were selected for confirmation. BTx378, BTx399, and IS22253, the contrasting TE lines reported by Peng and Krieg (1992) and Hammer et al. (1997) were included as controls. A high-

yielding commercial hybrid, Pioneer 84G62, and a publicly available hybrid, ATx623 × RTx430, and its parents were also included as controls. In addition, the two control lines, Liang Tang Ai and Tx7078, used in the initial survey were also included as controls. These 25 lines were studied under greenhouse conditions at Lubbock, TX (33°39'N, 101°49'W) in two successive experiments to confirm the lines selected from the prior survey experiments. The temperature was set at 28 °C day and 22 °C night. No supplemental light source was provided. The polyhouses used in the confirmation studies had no capability to regulate humidity. The actual temperature and relative humidity at plant level were monitored by a Hobo Pro RH/Temp series data logger (Bourne, MA). Plants were harvested at permanent wilt. The first confirmation experiment was conducted from May 9 to June 20, 2007. The maximum temperature was 34.7 °C, the minimum 19.1 °C, and the average was 26.3 °C. The average daytime VPD was 1.86 kPa. The second confirmation experiment was conducted from February 19 to April 4, 2008. The maximum temperature was 43.8 °C, the minimum temperature was 17.0 °C, and the average temperature was 27.1 °C. The average daytime VPD was 4.13 kPa. The increased VPD under late-winter greenhouse condition was likely due to the mode of temperature control and seasonal weather conditions. In the first experiment, the temperature was mainly controlled by evaporative cooling, providing a source of humidity, while in the second experiment, dry late-winter conditions required gas-heaters for temperature control, resulting in greater VPD.

2.5. Measurement of photosynthetic parameters

Physiological measurements in a greenhouse trial conducted at Colby, KS included gas-exchange instrumentation (LI-6400 with LI-6400-40 fluorometer, Li-Cor, Lincoln, NE) to quantify assimilation rate under standard conditions for the lines used in the confirmation studies. The TE protocol was similar to that described above, with the exception that seedlings were transplanted into pots that were maintained under well-watered conditions (relative water content of potting mix to exceed 70%). Measurement conditions included 1200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, 370 $\mu\text{mol CO}_2 \text{ mol}^{-1}$, 30 °C and VPD of $3.5 \pm 0.5 \text{ kPa}$. Measurements were made on the newest fully expanded leaf, after steady state conditions were obtained (indicated by fluorescence change of less than 5 quanta s^{-1} , steady stomatal conductance and assimilation rate); typically requiring a minimum of 15 min under standard conditions. Assimilation (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$), transpiration (T , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), VPD, stomatal conductance to water vapor (g_s , $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) and internal CO_2 concentration (C_i , $\mu\text{mol mol}^{-1}$) were calculated by manufacturer's software. Because the observed TE varies inversely to VPD, normalized TE (nTE, $\mu\text{mol CO}_2 \text{ kPa m}^{-1} \text{ H}_2\text{O}$), calculated as the product of the instantaneous TE (A/T) and VPD (Tanner and Sinclair, 1983), was used to compare the relation of TE with other photosynthetic parameters.

2.6. Experiment design and statistical analysis

A randomized complete block design with four replicates was used in both confirmation experiments. Analysis of variance and Means separation was performed on the lines using the GLIMMIX procedure in SAS (version 9.1.3) for the shoot and total TE variables. The block was treated as a random effect since no obvious pattern was observed. The LSMEANS option was used to calculate Tukey's HSD groupings of the lines (Littell et al., 2006). The means for dependent variables were rank ordered within each experimental location using the RANK procedure in SAS. The rank variables were analyzed by Wilcoxon Signed Rank (non-parametric paired t test) in the UNIVARIATE procedure in SAS. The

Wilcoxon signed Rank was used to determine if the relative rank in TE was different under the two different experiment conditions. For gas-exchange studies, A , T , g_s , C_i and nTE were analyzed by the MIXED procedure in SAS (version 9.1.3) as a randomized complete block design, using block as a random effect and VPD as a covariate, where significant. The LSMEANS option was used to calculate Tukey's HSD groupings of the lines. Normalized transpiration efficiency was regressed on C_i using the REG procedure of SAS (v 9.1.3). To determine the relative contribution of the amount of water transpired and dry biomass production to TE, correlation analysis between TE and water transpired or between TE and biomass production were analyzed separately.

3. Results

3.1. Initial survey of transpiration efficiency

The absolute TE values varied greatly from batch to batch and month to month depending on the ambient conditions (data not shown). During the entire survey, the TE_{total} for Liang Tang Ai varied from 5.1 to 8.9 g kg^{-1} water transpired; the TE_{total} for Tx7078 varied from 4.7 to 7.3 g kg^{-1} . In each batch of the survey, the TE was arranged from the highest to the lowest and separated into five ranks. Liang Tang Ai placed in rank 1 in 23 out of the 29 batches of the screen; Tx7078 placed in rank 5 in 20 out of the 29 batches of the screen. Despite the variation in TE from batch to batch, the relative rank of Liang Tang Ai was always higher than Tx7078, indicating the assay was quite reproducible. For comparison of the TEs from different batches, the TE value was expressed as the percentage to Liang Tang Ai in the same batch (supplement Table S1), which was set as 100%.

3.2. Variation of TE in selected sorghum accessions

The variation in TE among the 25 selected sorghum lines was confirmed under two different environmental conditions. In experiment 1, the average daytime VPD was 1.86 kPa; the average VPD in experiment 2 was 4.13 kPa. Both environment and line had significant effect on TE based on either shoot or total biomass ($P < 0.001$) (Table 1). The effect of environment and line on shoot biomass, root biomass, and water used were also significant at $P < 0.001$. The average TE based on total dry weight for experiment 1 was 10.8 g kg^{-1} ; it was 5.3 g kg^{-1} for experiment 2. The response of integrated TE based on biomass to VPD observed in this study is consistent to the response of instantaneous TE, the ratio of carbon dioxide assimilation to transpiration at the leaf level, to VPD (Tanner and Sinclair, 1983). Consistent with the difference in the experimental conditions used in the confirmation studies, environment had large effect on TE with F -value at 4632 and 4779 for TE based shoot biomass and total biomass, respectively. The effect of lines on TE with F -value of 6.69, significant at $P < 0.001$ level, was much smaller than the effect of environments. The $G \times E$ interaction was much smaller than the effect of line or environment. No significant effect of $G \times E$ for TE based total

Table 1
Analysis of variance of TE based shoot biomass and total biomass.

Effect	DF	F -value (TE_{shoot})	F -value (TE_{total})
Lines	24	13.92***	6.69***
Experiments	1	4632.65***	4779.42***
Line × Exp	24	3.31**	1.8

* Significant at 0.05 level.

** Significant at 0.01 level.

*** Significant at level < 0.001 .

biomass was detected. However, the effect of $G \times E$ for TE based shoot biomass was found significant.

Because the environmental conditions used in the two confirmation studies were greatly different and the main concern was the relative rank of the lines under the two conditions, Wilcoxon Signed Rank test was used to re-analyze the data. The P -value for the rank comparison of experiment 1 and experiment 2 was 0.9 for TE_{shoot} ; the P -value for TE_{total} was 0.86 indicating that no statistical difference in rank order of TE_{shoot} or TE_{total} was observed between the two experiments. The average TE from the two studies was presented in Table 2. The top five lines identified included PI391652, PI584085, Liang Tang Ai (a previously identified line for high TE control), PI291382, and PI567933; the bottom five lines included PI257309, BTx399, PI510898, PI586381, and Tx7078 (a previously identified line for low TE control). The average shoot and total TE for the top five lines were 6.6 and 8.8 $g\ kg^{-1}$, respectively, while the average shoot and total TE for the bottom five lines were 5.0 and 7.3 $g\ kg^{-1}$, respectively. Those lines displayed consistently high or low TE across different environments and should provide germplasm useful for investigations into the physiological mechanisms underpinning TE in sorghum.

3.3. Transpiration efficiency was correlated with increased biomass accumulation

TE, as used here, is the ratio of dry biomass produced to water transpired. A high TE value could result either from increased biomass produced for the same amount of water transpired, same amount of biomass produced with reduced transpiration, or a combination of both. To analyze which factor contributes more to TE in the lines identified by the gravimetric method, TE was regressed against the total amount of water transpired (Fig. 1A) or the total biomass produced (Fig. 1B) during the experiment. TE had little correlation ($r^2 = 0.07$) with water transpired but was highly correlated with the amount of biomass produced ($r^2 = 0.74^{**}$). This result indicates that increased biomass production, rather than

reduced water use, accounted for the increased TE among these sorghum lines. This contrasts with transpiration control as a factor in TE for the lines selected based on carbon isotope discrimination methods in other plant species (Condon et al., 2002; Impa et al., 2005).

3.4. TE is associated with low internal CO_2 concentration and maximum photosynthetic capacity in some high TE lines

Gas-exchange measurements indicated significant effects of genotype on nTE , C_i , g_s , A and T (Table 3). The line with the greatest g_s (PI 295121) also registered the greatest A and T ; contrasting lines with small g_s (PI 391652 and PI 236278) also tended to have small values of A and T . Six of the seven lines with lower C_i had significantly greater nTE than the lines with higher C_i . Normalized transpiration efficiency was strongly related ($r^2 = 0.903$) to the ratio of C_i to ambient CO_2 concentration (C_a) (Fig. 2).

4. Discussion

In this report, we have identified a set of sorghum accessions with high and low TE from 341 sorghum PI lines and breeding materials. The TE based on biomass accumulated per unit water transpired is a highly variable trait. It is greatly influenced by VPD, which fluctuates with changes in temperature, wind speed, and relative humidity. Indeed, TE reported from the literature ranged from 2.8 to 12.6 $g\ kg^{-1}$ (Donatelli et al., 1992; Peng and Krieg, 1992; Hammer et al., 1997; Henderson et al., 1998; Mortlock and Hammer, 1999; Briggs and Shantz, 1913). Even TE for the same line varies from report to report (Mortlock and Hammer, 1999). Similar variation in TE was observed throughout the initial surveys in this study: the range in TE was 43% of the mean for Tx7078 and 54% of the mean for Liang Tang Ai. Thus, it is difficult to compare the absolute TE reported from different studies. However, we showed here that it is possible to compare the relative TE based on a standard control that is included in each experiment.

Table 2

Variation of transpiration efficiency based on shoot dry weight (TE_{shoot}) and total dry weight (TE_{total}) in 25 selected sorghum lines.

Lines	Shoot DW (g)	Root DW (g)	Water used (g)	TE_{shoot} ($g\ dw\ kg^{-1}$)	TE_{total} ($g\ dw\ kg^{-1}$)	Rank based on TE_{total}
PI391652	5.8	1.5	825.4	7.1 a	8.9 a	1
PI584085	4.9	2	745.6	6.3 abcde	8.9 a	2
LiangTangAi	5.8	1.8	871.2	6.6 abc	8.8 ab	3
PI291382	5.8	1.7	859.6	6.7 ab	8.6 abc	4
PI567933	5.3	1.8	816.1	6.5 abcd	8.6 abc	5
PI533946	4.8	2.2	849.2	5.7 defghi	8.3 abcd	6
PI295121	4.5	2.3	828.6	5.5 fghij	8.3 abcd	7
PI236278	4.4	2.3	812.6	5.4 fghij	8.3 abcd	8
PI267392	4.4	1.9	734.6	5.7 defghi	8.2 abcd	9
IS22253	4.9	1.8	811.6	5.9 bcdefg	8.2 abcd	10
PI262568	4.7	1.9	812.5	5.9 bcdefgh	8.2 abcd	11
BTx378	4.7	1.6	766.3	6.0 bcdef	8.1 abcd	12
PI563588	4.9	1.7	815.3	6.0 bcdef	8.1 abcd	13
Pioneer84G62	4.6	1.8	795.9	5.9 cdefgh	8.1 abcd	14
PI534138	4.5	2.1	814.7	5.5 efghi	8.1 abcd	15
BTx623	4.4	2.2	818	5.4 fghij	8.1 abcd	16
PI267532	4.3	1.8	735.5	5.5 efghij	7.9 abcde	17
ATx623XRTx430	4.3	2.1	815.1	5.3 fghij	7.9 abcde	18
PI276797	4.8	1.7	828	5.8 defghi	7.9 abcde	19
RTx430	4	2	745.5	5.1 ghij	7.8 bcdef	20
PI257309	4.3	2.1	828.4	5.2 ghij	7.7 bcdef	21
BTx399	4	2	757.7	5.1 hij	7.7 bcdef	22
PI510898	4.5	1.9	864.6	5.3 fghij	7.5 def	23
PI586381	3.7	1.8	762.1	4.7 j	7.0 ef	24
Tx7078	4.1	1.5	836.1	5.0 ij	6.8 f	25

Tukey-Kramer grouping (Littell et al., 2006) of the inbred and hybrid lines using least square means option in GLIMMIX procedure (SAS version 9.1.3). LSMEANS estimates with the same letter are not significantly different.

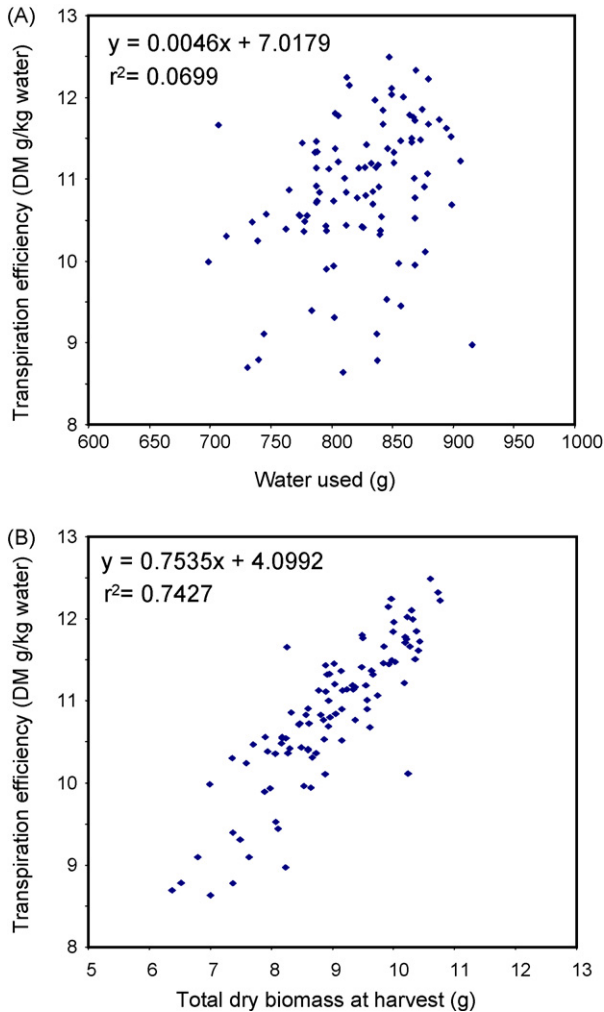


Fig. 1. Relation between transpiration efficiency based on total dry weight to total water used and total biomass produced.

To provide a comparison with the previously reported result, three accessions that are available were included in our confirmation studies. Peng and Krieg (1992) identified BTx378 and BTx399 as high and low TE lines, respectively. In our experiment, BTx378 ranked 12 among the 25 lines used, while BTx399 ranked 22. Another line IS22253, reported to have superior

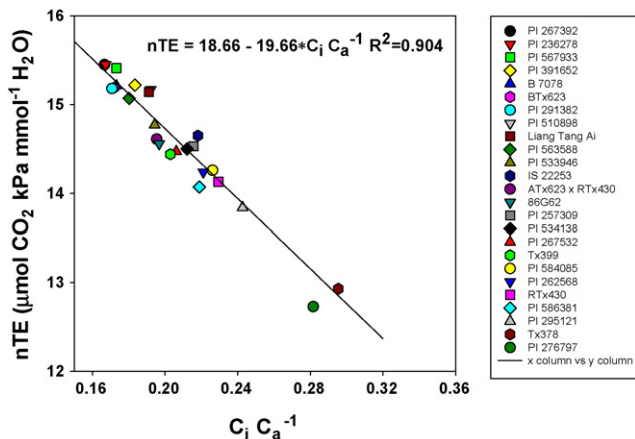


Fig. 2. Relationship of instantaneous transpiration efficiency normalized by VPD (nTE) with the ratio of internal CO₂ concentration to air (C_i/C_a).

TE (Hammer et al., 1997), ranked 10 among the lines used in this study. It should be noted that the lines selected for the confirmation study were based on their relative TE to Liang Tang Ai from one experiment. All the bottom five lines had a relative TE below 75% of Liang Tang Ai in the original survey. Four of the high TE lines had a relative TE around 100% or higher. One high TE line, PI567933, had a relative TE at 69% of Liang Tang Ai in the original survey but ranked to top five lines in both confirmation tests. The reason for this is unclear; but it could occur from many conceivable factors, such as soil borne diseases not immediately visible, bad quality of plastic membrane, and seed contamination or variation. Thus, discrepancy between the original survey and the re-confirmation tests could occur due to uncontrollable experimental errors that might happen during the initial survey. Nevertheless, several sorghum accessions with higher or low TE were identified through re-confirmation tests. These sorghum accessions, together with the previously identified lines, should provide critical materials for studying the physiological mechanisms of TE. Those lines are available for distribution by contacting the authors or ordered on-line from the National Germplasm Resources Information Network of Agricultural Research Service of the United States Department of Agriculture (<http://www.ars-grin.gov/>).

Sorghum is usually grown under rain-fed conditions with no or limited irrigation. Its yield is strongly influenced by the availability of soil water throughout the growing season. The TE based on integrated biomass, the amount of dry biomass accumulated per unit water transpired, may be a relevant trait to enhancing sorghum yield under water-limited conditions. The enhanced TE may benefit sorghum production in two important aspects. On one hand, high TE allows sorghum plants to accumulate more biomass, and possibly higher grain yield if harvest index remains the same, from the same amount of soil water available to the plant during the growing season. On the other hand, high TE may allow sorghum plants to survive longer with the same limited amount of soil water and possibly avoid a detrimental stress before the next rain. Thus, it is conceivable that a small improvement in TE may translate to a meaningful gain in yield. Indeed, wheat varieties selected for TE with a small improvement in $\Delta^{13}\text{C}$ discrimination ratio from 21.6‰ to 21.2‰ on average produce significantly higher biomass and grain yield, especially under low rainfall conditions (Rebetzke et al., 2002). In our experiment, the average TE of the top five lines was about 20% higher than the average of the bottom five lines. Apparently, enhancing TE through breeding may have a large potential to increase sorghum yield in arid and semi-arid regions with no or limited irrigation.

Photosynthetic discrimination against stable carbon isotope $^{13}\text{C}_2$ over CO_2 ($\Delta^{13}\text{C}$) has been correlated with transpiration efficiency in C_3 plants (Farquhar et al., 1982; Scheidegger et al., 2000; Condon et al., 2002; Lambrides et al., 2004; Impa et al., 2005; Stiller et al., 2005). In general, high TE lines selected by reduced $\Delta^{13}\text{C}$ discrimination is often associated with reduction in transpiration, slow growth, and reduced biomass production under nonstressed conditions (Condon et al., 2002, 2004; Impa et al., 2005). Nevertheless, wheat lines selected with low $\Delta^{13}\text{C}$ have significant advantage in grain yield and biomass production under rain-fed conditions (Condon et al., 2002). However, carbon isotope discrimination method cannot be easily applied to C_4 plants (Hattersley, 1982; Farquhar, 1983). The $^{13}\text{C}/^{12}\text{C}$ isotope ratio is not a fixed value in C_4 species, and its value depends on how far the leaf Kranz anatomy is developed, on the degree of leakiness of the bundle sheath cells for CO_2 , and on the separation of the C_4 PEP carboxylase from the C_3 Rubisco (Hattersley, 1982). The carbon isotope discrimination ratio has not been used successfully to identify C_4 plants with high TE, as it has been used in C_3 plants (Mortlock and Hammer, 1999).

Table 3

Variation of normalized transpiration efficiency (nTE), leaf internal CO₂ concentration (C_i), stomatal conductance to water vapor (g_s), assimilation (A), and transpiration (T) based on gas-exchange measured under standard conditions in 25 selected sorghum lines.

Lines	nTE $\mu\text{mol CO}_2 \text{ kPa mmol}^{-1} \text{ H}_2\text{O}$	C _i $\mu\text{mol mol}^{-1}$	g _s $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$	A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	T ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)
PI267392	15.45 a	61.7 c	0.181 ab	30.9 ab	5.99 ab
PI236278	15.45 a	61.9 c	0.143 b	25.1 ab	4.99 ab
PI567933	15.41 ab	64.1 bc	0.180 ab	29.3 ab	5.25 ab
PI391652	15.22 ab	67.9 bc	0.152 b	25.6 b	4.99 b
Tx7078	15.21 ab	64.1 bc	0.221 ab	36.3 ab	7.26 ab
BTx623	15.19 ab	63.6 bc	0.181 ab	30.0 ab	6.06 ab
PI291382	15.18 abc	63.2 bc	0.174 ab	29.9 ab	5.67 ab
PI510898	15.17 abc	71.1 abc	0.184 ab	29.0 ab	5.56 ab
LiangTangAi	15.14 abc	70.7 abc	0.183 ab	29.1 ab	5.96 ab
PI563588	15.07 abc	66.7 abc	0.166 ab	28.4 ab	5.68 ab
PI533946	14.77 abc	71.9 abc	0.225 ab	34.7 ab	7.46 ab
IS22253	15.65 abc	80.7 abc	0.182 ab	28.8 ab	5.98 ab
ATx623XRTx430	15.61 abc	72.3 abc	0.237 ab	37.1 ab	7.58 ab
Pioneer84G62	14.56 abc	72.8 abc	0.205 ab	33.4 ab	6.48 ab
PI257309	14.53 abc	79.8 abc	0.199 ab	30.5 ab	6.17 ab
PI534138	14.50 abc	78.5 abc	0.176 ab	28.8 ab	6.19 ab
PI267532	14.47 abc	76.3 abc	0.219 ab	34.2 ab	6.77 ab
BTx399	14.44 abc	75.1 abc	0.219 ab	34.8 ab	6.86 ab
PI584085	14.26 abc	83.7 abc	0.196 ab	30.9 ab	6.62 ab
PI262568	14.24 abc	81.8 abc	0.206 ab	32.3 ab	6.69 ab
RTx430	14.13 abc	84.9 abc	0.238 ab	34.7 ab	7.36 ab
PI586381	14.07 abc	81.0 abc	0.198 ab	30.8 ab	6.03 ab
PI295121	13.84 abc	89.8 abc	0.263 a	38.8 a	8.20 a
BTx378	12.93 bc	109.3 a	0.214 ab	30.6 ab	6.84 ab
PI276797	12.73 c	104.2 a	0.227 ab	32.6 ab	6.89 ab
Effect of VPD (coefficient with VPD as a covariate)	n/a	-8.94	n/a	2.51	2.42

Tukey's Studentized Range (HSD) grouping (Littell et al., 2006) of the inbred and hybrid lines using the LSMEANS option in Proc MIXED (SAS version 9.13), adjusted for vpd effects. LSMEANS estimates with the same letter are not significantly different.

In order to select sorghum lines with high TE, we configured a high throughput method to screen TE based on biomass accumulation for a given amount of water transpired at early vegetative stage (Xin et al., 2008). Several lines with high or low TE were identified. Impa et al. argued that high TE can be achieved either through reduction in transpiration rate or through enhanced photosynthetic capacity (Impa et al., 2005). Therefore, we analyzed the correlation of TE with biomass accumulation or water use independently. The TE determined by biomass accumulation per unit water transpired was positively correlated with biomass production (Fig. 1). Little correlation between TE and total water use was identified. Thus, the high TE lines selected by biomass accumulation were due to superior productivity rather than relative reduction in water use that is often associated with the lines selected for reduced $\Delta^{13}\text{C}$ discrimination ratio in C₃ plants. Our study suggests that TE based on biomass accumulation may be a relevant approach to determine and select for high TE lines in sorghum.

We began to use these sorghum lines with different TE to study the physiological processes contributing to TE. Gas-exchange measurements demonstrated that the lines with significantly reduced g_s also exhibited significantly smaller C_i values. The role of stomatal conductance in regulation of A and T is well recognized (Cowan and Farquhar, 1977), though incompletely defined (Jones, 1998). Thus the corresponding differences among the lines in g_s, A and T conform to current knowledge of stomatal function. However, C_i represents a related factor which affects the ratio of A:T and nTE at the leaf level. The negative linear relationship between C_i and nTE, illustrated in Fig. 2, can be derived from the flux-gradient equations for A and T, specified relative to the boundary conditions across the stomatal cavity. For two leaves with equivalent vapor pressure gradients and stomatal conductances, the leaf with the least C_i/C_a will maintain greater assimilation, and thus greater transpiration efficiency, at the leaf-level. Thus, C_i represents a useful surrogate for assimilation and transpiration efficiency processes (Von Caemmerer and Furbank, 1999).

Overall, little relation was found between TE based biomass production integrated over time with the instantaneous TE determined at leaf level. However, two lines (PI 391652 and PI 291382) with significantly smaller values of g_s, A and T, but significantly greater values of nTE also displayed higher TE based biomass production (ranked 1 and 2 in TE_{shoot} and 1 and 4 in TE_{total}, respectively). However, other lines (IS22253, PI563588, PI267392, and PI586381) that exhibited restrictive stomata, i.e., reduced C_i and greater nTE, did not exhibit high TE based biomass production. Thus, lines with putative TE traits at the leaf level can exhibit superior TE, evaluated at the whole-plant level; but not in every case. Our results indicate that other physiological processes in addition to the instantaneous TE at leaf level also contribute significantly to the TE based on integrated biomass production. The sorghum lines identified in this study may help to elucidate these mechanisms.

Root growth, distribution, and function could alter assimilation and utilization processes (Aiken and Smucker, 1996), the balance of which could alter TE. Hammer et al. (1997) reported that the root fraction of total biomass varies greatly among sorghum lines, and neglect of which could alter TE rankings among lines. In this study, the differential ranking of lines with respect to TE_{shoot} and TE_{total} is also attributed to differential root fraction. In addition to representing a substantial sink for assimilates and the origin of the transpiration stream, root systems can originate signals affecting stomatal conductance (Davies and Zhang, 1991) and shoot growth (Masle and Passioura, 1987).

Hybrid sorghum cultivars provide a large advantage over pure lines in grain yield and adaptation to environmental stresses. Hybrid cultivars have become the predominant cultivars used commercially in the United States since its introduction in the 1950s (Rooney, 2004). To provide a comparison of the TE of the selected PI lines with sorghum hybrids, a high-yield commercial hybrid Pioneer 84G62, a public hybrid ATx623 × RTx430 and its parents were included as controls. The public hybrid ranked the 18th place in our experiments; and its parents ranked in 16th and

20th places, respectively. The high-yield commercial hybrid Pioneer 84G62 ranked the 14th place in the 25 lines used in this study. The TE performance of its parents is unknown since this information is unavailable for the commercial hybrid. Further study is required to determine if high TE traits of individual pure lines can be expressed in hybrids. Effort is under way to create isogenic lines of elite breeding materials that differ only in TE. Those lines will be used to determine if high TE can be expressed in hybrids under field conditions. Furthermore, we are developing recombinant inbred lines derived from crosses between lines with contrasting TE for mapping quantitative trait loci mediating TE. Identification of these loci may help ingress this trait into elite lines via molecular marker assisted breeding.

5. Conclusions

Screening of sorghum PI accessions, followed by confirmation studies, identified several lines with reproducible differences in TE. Instantaneous TE at leaf level derived from gas-exchange analysis was negatively correlated with internal CO₂ partial pressure. Leaf level TE can explain the biomass-based TE integrated over the entire growth period in some but not all lines, indicating that mechanisms other than stomatal control also contribute to the TE based on biomass production. The sorghum accessions identified in this study provide critical materials to study the physiological mechanisms and mode of inheritance of biomass-based TE. The challenge is to express the high TE traits identified in the PI lines in sorghum hybrids that can be used in commercial production.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.fcr.2008.10.010](https://doi.org/10.1016/j.fcr.2008.10.010).

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