DETERMINING TRANSPIRATION EFFICIENCY OF EIGHT GRAIN SORGHUM LINES [*Sorghum bicolor* (L.) Moench]

By

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Abstract

Transpiration efficiency (TE) is defined as total biomass produced per unit of water transpired. Improvement of TE means maximizing crop production per unit of water used. The objectives of the study were to examine, at the leaf level and the whole plant level, the variation in TE for sorghum [*Sorghum bicolor* (L.) Moench] accessions, previously screened for TE and to test physiological mechanisms that may account for differences in TE. Three field studies and two mini-lysimeter studies (one done in pots under greenhouse conditions and one done in pots in the field) were conducted with eight accessions. Instantaneous measurements of assimilation (A), stomatal conductance (g_s), and transpiration by gas exchange provided measures of the transpiration efficiency at the leaf level. Growth observations and soil water balance in field plots quantified components of whole-plant TE. Growth and development measurements showed significant difference, explaining the existence of photoperiod sensitivity among the sorghum genotypes. Assimilation (A), stomatal conductance (g_s), and maximum quantum efficiency of photosystem II (Fv/Fm) were consistently greater for accession PI533946 (from India) and greater for accession PI295121 (from Australia) in both field and the field-pot studies ($p<0.05$). Internal carbon dioxide (C_i), an indicator of intrinsic transpiration efficiency, differed among lines under field conditions ($p<0.05$). Leaf relative water content (RWC), measured in the greenhouse, and did not differ among the eight accessions. No consistent differences in biomass and water use were detected among lines under field conditions. In conclusion, developing reliable selection indices for TE will require a greater understanding of whole-plant physiological processes to utilize the differences in TE observed at the leaf level.

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Definition of symbols

Symbol	Unit	Definition
A	$\mu mol\ m^{-2}\ s^{-1}$	Assimilation of CO_2 per unit leaf area
Ci	$\mu mol\ mol^{-1}$	Intercellular carbon dioxide concentration
T	$mmol\ m^{-2}\ s^{-1}$	Transpiration
g_s	$mol\ m^{-2}\ s^{-1}$	Stomatal conductance
No abbreviation	s/cm or s/m	Stomatal resistance
ØPSII/ØCO_2	$mol\ ^{e-}\ mol\ CO_2^{-1}$	Quantum Yield Ratio
Fv/Fm		Maximum quantum yield of PPSII (Photosystem II)
TE	g/Kg	Biomass-based transpiration efficiency
CWU	cm	Cumulative water use
RWC	%	Leaf relative water content

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Dedication

I dedicate my thesis to my mother, Panchavarnam Ayyaru Thevar, who took hardship and care in raising me and my sisters.

CHAPTER 1 - Introduction

Transpiration efficiency

Water use efficiency is defined as the primary production of biomass (frequently limited to above-ground biomass or grain yield per unit of water use. Assuming that runoff and deep drainage are negligible, crop water use includes transpiration and evaporation from the soil. If soil evaporation can be prevented, water loss is only by transpiration and the biomass or grain yield per unit of transpiration is termed the transpiration efficiency (Fischer 1981). Passioura (1977) proposed that yield is a function of transpiration. Transpiration efficiency (TE) is defined as the biomass production per unit of water transpired and harvest index is defined as the ratio of grain mass to above-ground biomass. Instantaneous measurements of photosynthesis and transpiration by gas exchange give a measure of the transpiration efficiency at the leaf level under the precise conditions at the time of measurement. Integrated measures of transpiration efficiency can be calculated from biomass accumulation and water use by transpiration over days or weeks.

Species that were subsequently shown to have the C_4 pathway of photosynthesis had higher transpiration efficiencies than those with the C_3 pathway of photosynthesis (Briggs and Shantz 1912; Fischer and Turner 1978). While C_4 species tend to have a higher temperature optimum and grow in the warmer periods of the year with high vapor-pressure deficits, the selection of genotypes with the ability to grow in cooler temperatures has allowed them to be grown in temperate regions, where their higher transpiration efficiency can result in higher yields than C_3 species on the same amount of rainfall. Thus, choice of species can be used to improve yields with similar water use, that is, to increase the rainfall-use efficiency. For example, (Jones and Popham (1997) showed that growing sorghum (*Sorghum bicolor* (L.) Moench) rather than wheat (Triticum aestivum L.) more than doubled the grain yield and increased precipitation-use efficiency (maximization of biomass production per unit of precipitation received) in the western plains of the United States of America.

Environmental factors affecting TE

Transpiration efficiency varies with crop type and atmospheric humidity, with higher efficiencies in more humid environments. In principle, therefore, more biomass could be produced using the same amount of water by selecting species with high transpiration efficiencies or by growing plants in more humid air. The latter could be done on a macro scale, that is, by growing plants and/or using irrigation water at times, or in places, where air humidity is high. There is also some scope for microclimate manipulation in semi-arid regions where the relative humidity around crops can be increased using an over-storey of trees.

Tanner and Sinclair (1983) concluded that a significant improvement in TE is unlikely. Ritchie (1983) considered that improving crop management, i.e., practices under the control of the farmer, can lead to an increased TE. Improving poor crop management may lead to the attainment of a maximum TE, but further attempts at improving management have no effect. Subsequent studies have identified genetic variation in transpiration efficiency within a species, example include peanut (Arachis hypogaea L.) (Hubick et al.1986) wheat (Farquhar and Richards 1984) and sorghum (Hammer et al. 1997). However, genetic improvements can increase the currently accepted maximum value of TE, (Condon et al.2000).

The other factor leading to increased TE is the increase in the atmospheric CO_2 concentration (Polley 2000). In the 17 yr since Tanner and Sinclair (1983) concluded that TE is a stable parameter, the level of CO_2 in the atmosphere has risen from 335 to 360 ppm. For C3 species, TE is proportional to the CO_2 gradient from the atmosphere to the mesophyll, which is about 70 ppm (Condon et al. 2000). The proportional change in the CO_2 gradient between 1983 and 2000 is given by

$$(C_{a,2000} - C_{a,1983})/(C_{a,1983} - C_i)$$

Where $C_{a,2000}$ is the atmospheric CO_2 concentration in 2000, $C_{a,1983}$ is the atmospheric CO_2 concentration in 1983, and C_i is the CO_2 concentration in the mesophyll. The proportional change in the CO_2 gradient between 1983 and 2000 would lead to a 10% increase in the TE. This is supported by recent unpublished observations in southeastern Australia where TE is >22 kg ha^{-1} mm^{-1}, rather than equal to 20 kg ha^{-1} mm^{-1} as reported by French and Schultz (1984)

Genetic variation in TE

Understanding how growth and development of grain sorghum genotypes respond to water limitation would provide a basis to assess the value of such responses in crop production and crop improvement. At the individual leaf level, the ratio between CO_2 assimilation rate (A) and stomatal conductance (g_s), A/g_s gives the intrinsic TE (Condon et al. 2000); these parameters are also indicators of intrinsic differences in productivity and water use efficiency (WUE), which has been demonstrated in grain sorghum [*Sorghum bicolor* (L.) Moench] hybrids. Several authors provide information on genetic variation for A and g and their response to environment. Blum and Sullivan (1972) and Krieg and Hutmacher (1982) reported genetic variation for A while Henzell et al.(1976) and Hofmann et al.(1984) presented evidence for genotype differences for g_s. Krieg and Hutmacher (1982) have reported that A, g_s, and A: g_s ratio vary in a single sorghum hybrid due to plant age and water stress. Pre-flowering leaf photosynthetic rate of sorghum has been found to be correlated with biomass and grain production under both well-watered and water- limited conditions (Peng and Kraig et al.1992). The rate of CO_2 fixation (A) is highly correlated with stomatal conductance (g_s), but the ratio of A to g, and of biomass production to crop transpiration, have been shown to be affected by both environment and genetics in C_4 plants (Kidambi et al.1990). Genetic differences for A/g_s were convincingly demonstrated by Kidambi et al. (1990), who concluded that genetic differences in variation in intrinsic water- use efficiency could directly contribute to increased whole plant water-use efficiency and productivity. Other physiological mechanisms may account for genetic differences in TE.

The maintenance of a high photosynthetic activity and efficient gas exchange of the uppermost leaves and internodes under environmental limitations may increase the efficiency of water use of the whole plant canopy, providing adaptive value (Richards 1993, Wojcieska 1994, Starck 1995, 2001). Therefore, breeding cultivars more efficient in carbon dioxide assimilation and whole-plant water use could increase transpiration efficiency. Limitations on maximum transpiration rates, which are commonly observed as midday stomatal closure, have been observed even under well-watered conditions (Sinclair et al. 2005). Such limitations may be caused by restricted hydraulic conductance in the plant or by limited supply of water to the plant from uptake by the roots. This behavior would have the consequences of limiting photosynthetic rate, increasing transpiration efficiency, and conserving soil water.

Relationship of TE to drought avoidance, drought tolerance

Increasing WUE of sorghum could improve food security, since it is grown in semiarid parts of the world where water supply is the primary limitation to productivity. Improvement of TE means maximization of crop production per unit of water use (Turner et al. 2001). Sorghum genotypes were found to differ for nearly all recognized drought resistance mechanisms (e.g., Blum 1979; Sullivan and Ross 1979), such as: maintenance of high leaf water potential and stress, deeper root growth, stomatal control over transpiration, osmotic adjustment, and carbon fixation. There are physiological mechanisms which could account for the genetic difference in TE involving relative water content and transpiration control. Under stress conditions, plants with high relative water content may transpire less due to reduced stomatal conductance, thus restricting water loss by transpiration (Barrs and Weatherley 1962).

Physiological mechanisms at the leaf level can affect photosynthetic activity. For example, genotypes differing in C_i can alter the relationship of assimilation and transpiration. Leaves maintaining smaller C_i will have a stronger CO_2 driving gradient; this can result in greater transpiration efficiency, relative to leaves with greater C_i, if the stomatal conductance's and vapor pressure deficits are equivalent.

(Turner et al. 1984) showed that increasing the vapor pressure deficit of the atmosphere surrounding a leaf can result in decreased leaf conductance, net photosynthesis and leaf water potential. If genotypes show variability in conductance response to vapor pressure deficit of the atmosphere, then screening genotypes for these differences can help selection of genotypes suitable for drought conditions. Similarly, plant productivity is governed by the ability to absorb and utilize photosynthetic active radiation. Leaves are the primary organs for light absorption. Leaf orientation and angle can affect light absorption efficiency. More efficient light absorption by leaves can increase assimilation and plant growth—particularly during canopy development.

The function of the unique reactions of C_4 photosynthesis is to concentrate CO_2 in bundle-sheath cells for assimilation via Rubisco (Hatch 1987). Any CO_2 that leaks from bundle-sheath cells into mesophyll cells will be assimilated by PEP carboxylase a second time, reducing the efficiency of C_4 photosynthesis. Thus, bundle sheath leakage of CO_2 reduces assimilation efficiency and primary productivity. Plant growth is the balance of photosynthetic gains and respiratory losses, therefore respiration can affect plant productivity. Carbon lost through

respiration can account for up to 50% of the daily carbon gain by photosynthesis (Morgan and Austin 1983). McCree (1974) concluded that grain sorghum (C_4) was lower in respiration coefficient than white clover (C_3). Considering that these physiological mechanisms may differ among genotypes, several hypotheses were identified which could account for differences in TE among sorghum lines.

Research hypothesis

Physiological mechanisms hypothesized to account for differences which may be observed in TE among sorghum germplasm are:

- Increased relative water content and stomatal conductance result in decreased leaf transpiration and increased TE.
- Increased leaf respiration would cause the plant to increase the consumption of assimilates and reduce potential biomass accumulation, reducing TE.
- Decreased leaf internal carbon dioxide would increase transpiration efficiency due to reduced transpiration ratio.
- Increased leaf angle and leaf orientation would increase light penetration into the vegetative canopy with subsequent increase in leaf CO_2 assimilation for increased TE.
- Bundle sheath leakage of CO_2 would decrease light use efficiency and biomass accumulation, resulting in reduced TE.
- Plants which reduce stomatal conductivity at greater vpd, relative to that of lesser vpd, will reduce transpiration and may increase TE (depending on assimilatory effects).

The objectives of the study were to examine, at leaf level and whole plant level, the variation in TE for sorghum accessions, previously screened for TE and to test physiological mechanisms which may account for differences in TE.

CHAPTER 2 - Evaluation of Transpiration Efficiency of Eight Grain Sorghum Lines

Greenhouse Study – Materials and Methods

The experiment was carried out between 3 December 2006 and 16 February 2007 in a greenhouse at Kansas State University in Manhattan, KS (39°08'N, 96°37'W, 314 m above sea level).

On 3 December (Day 0), 6 to 8 seeds of each sorghum genotype (Table 2.1) were placed in a 100 mm (diameter) x 15 mm (height) sterile Petri plate. The seeds had been sterilized by soaking them in one-third concentration Clorox in a beaker for 15 minutes. Before being placed in the Clorox solution, the seeds had been dipped in 70% alcohol to break the surface tension. They were washed with tap water thoroughly after being removed from the Clorox solution. By 6 December, radicals had emerged on the seeds and on this day six seeds of each genotype were placed at the 2.5 cm depth in one of 48 black plastic pots (16 cm diameter; 18 cm height) with drainage holes, which had less covered with a paper towel.The procedure of Xin et al. (2008) then were followed. The pots were filled with a commercial potting mixture (Sunshine Mix No. 1, Bellevue, WA, USA). It has a composition of 70 to 80% Canadian peat moss, perlite, dolomite, limestone, and gypsum added as a wetting agent. The pots had been filled with the mix on 1 Dec. 2006, after which, they were watered with a fertilizer solution. The fertilizer (Miracle-Gro Products, Inc., Port Washington, New York) had a composition of 15N:30P_2O_5:15K_2O, and 0.5 gram of the fertilizer was dissolved in one liter of de-ionized water to give the desired concentration of the elements. The pots were placed on a tray (four pots per tray) and drenched once. The trays with pots then were slanted at an angle, and the pots were left to drain for 24 hours. The water content in the pots was at pot capacity when the seeds were planted. Day zero is defined as the day of emergence. Emergence was recorded Day 3, 4, 5, 8, 12, and 16. After emergence was recorded on Day 16, each pot was thinned to three seedlings per pot.

On 15 December (Day 12 after germination began), the pots were wrapped in plastic (8 mil poly bags, Uline Plastic, Waukegan, IL, USA). Two bags were used for each pot (Xin et al., 2008). The bottom of a pot was put in the bottom of one plastic bag. Another plastic bag was

put over the top of a pot. Holes were cut in the plastic to allow the seedlings to protrude through the holes. The plastic was secured to the pot by putting a rubber band around the exterior circumference of the pot. The plastic prevented evaporation from the soil, so that water lost from the pot was due to transpiration.

The two watering regime were watered an un-watered. Water was added by placing a funnel inside the hole in the plastic where a plant emerged. This way, water could be added to the soil surface without disturbing the plastic. On 26 Dec. (Day 23) and 30 Dec. (Day 27), approximately 100 mL water was added to each pot in both the watered un-watered treatments. On 3 Jan. 2007 (Day 31), another 100 mL was added to each pot in the un-watered treatment. No more water was added to the pots in the un-watered treatment after Day 31. Plants in the watered treatment were watered on 6 Jan. (Day 34), 9 Jan. (Day 37), 13 Jan. (Day 41), 29 Jan. (Day 57), and 8 Feb. (Day 67), each time with about 100 mL per pot. Thus, after the initial drenching of the pots with the fertilizer solution, each watered treatment pot received about 700 mL of water and each un-watered treatment pot received about 300 mL water. Pots were weighed before and after watering on each day water was added. Total water used by each pot was determined by taking the difference in weight after watering on one day and that measured before watering at the next watering time. For example, PI257309 weighed 997 grams after watering on 26 Dec. and 889 grams before watering on 30 Dec. It lost 108 grams (mL) of water during that time period (26 to 30 Dec.). These time periods were added up to get the total water used by plants in each pot during the entire experiment (from watering to pot capacity on 1 Dec. 2006, which was two days before Day 0, the beginning of germination, to the last watering time on 8 Feb. 2007 or Day 67).

Relative water content was determined by using the method of Barrs and Weatherley (1962). Relative water content was measured on Days 51, 61, and 75. These days were chosen because they corresponded approximately to the fifth, seventh, and eighth leaf stages (Vanderlip 1972; Vanderlip and Reeves 1972). One recently matured leaf was cut from a plant and then cut in half along the length of the leaf (next to the main vein). One half of each leaf then was cut into 10 segments. The other lengthwise half of the leaf was discarded. The fresh weight of these segments was determined. The segments were placed in a Petri plate with tap water and floated for 24 hours on a laboratory bench. The turgid weight of the segments was determined after blotting them dry with tissue paper, and then the segments were dried in an oven at 80^{o}C for 48

hours and dry weight was determined. Relative water content was calculated as follows: (fresh weight minus dry weight)/ (turgid weight minus dry weight).

Height of all shoots in each pot was measured on 18 Jan. (Day 46) and at harvest (16 Feb., Day 75). As soon as the leaves were large enough to measure stomatal resistance, abaxial stomatal resistance was measured on 10 different days (Day 31, 33, 35, 37, 39, 41, 43, 46, 48, and 50) starting at 10:00 h with a steady state diffusion porometer (Model SC-1, Decagon Devices, Pullman, WA, USA). The diffusion porometer can be set to measure either stomatal resistance or stomatal conductance. The porometer was set to measure stomatal resistance to emphasize the fact that it is a true (physical) resistance that is measured. The more closed the stomata are, the higher is the resistance that water molecules encounter as they move from the substomatal cavity into the air. Because porometers were developed by physicists, the early literature (and some modern literature) gives data from porometers in resistance units. The current literature usually presents stomatal conductance, which is the reciprocal of stomatal resistance (conductance = 1/resistance). Conceptually, it is easier to think of a conductance rather than a resistance. The units of stomatal conductance are either cm/s (or m/s) or mmol m^{-2} s^{-1}. Stomatal resistance was measured on the abaxial surface, because stomatal density is higher on abaxial surfaces than on adaxial surfaces of sorghum (Liang et al. 1975). The most recently matured leaves on each of the three plants in each pot were measured. Measurements also were taken occasionally on the second most recently matured leaf, so that on some days more than three measurements per pot were taken. The sensor head of the porometer contains two humidity sensors: one close to the leaf and one farther away from the leaf. Temperature is recorded at these two locations, too. In addition to the stomatal resistance, these four values are displayed on the read-out of the porometer (relative humidity at two locations; temperature at two locations). The porometer calculates stomatal resistance from these measured values of temperature and relative humidity (Decagon Devices 2006). All these values were recorded when a stomatal resistance reading was taken. Only the stomatal resistance will be reported in this paper.

At harvest (16 Feb. 2007) shoots were cut and fresh weights were determined. None of the plants had reached reproductive stage by harvest, so only vegetative matter was harvested. Shoots were dried at 80 ^{o}C for 48 h and dry weights were recorded. At harvest, roots were extracted by turning each pot upside down. The soil came out in one clump. The soil in each pot

was placed over a 1 mm mesh sieve and the soil was washed away. Roots that were trapped by the sieve were dried at 80 ^{o}C for 48 h and dry weights were determined.

Pressure potential was determined at harvest (Day 75) on the most recently matured leaf using a portable plant water status console (Model 3115, Soil moisture Equipment Corp., Santa Barbara, CA, USA).

During the experiment, the temperature and relative humidity were monitored with a data logger (HOBO Model No. HO8-004-02, Onset Computer Corporation, Bourne, Massachusetts). Minimum night temperatures ranged between 15 and 21 ^{o}C, and maximum day temperatures ranged between 21 and 37 ^{o}C. Relative humidity ranged between 25 and 95%. Natural daylight was used during the experiment.

The experiment was a completely randomized block design with eight sorghum lines, two treatments (wet and dry), and three replications. Data were analyzed using Version 9.1.3 of Statistical Analysis Systems (SAS Institute, 2002-2003). Standard deviations and standard errors are presented in the figure and tables.

Field Lysimeter Study

Experiment and treatment design

Transpiration efficiency and physiological measurements were completed for selected sorghum lines (Table 2.1) during vegetative growth under field conditions at Colby, KS. The pot procedure of Xin et al. (2008) provided field lysimetric method placing the pots inside the green house. However the pots were placed outside in the field rather than inside the greenhouse. Each pot was placed in an excavation micro plot. The pots were surrounded with plants of the same genotype and growth stage, which were planted directly into the soil concurrent with planting in the pot. Pots were sheltered from precipitation by moving them to a greenhouse as conditions warranted. The pots were irrigated periodically to maintain well-watered conditions. Irrigation amounts were determined from mass of the pots before and after irrigation events. Once the pot reached below 700g of the total weight, it was re-watered for a value close to the range of 1000g of total weight. The experiment was a randomized complete block design with treatment as lines.

Growth and development

Allometric measurements (measurements of the morphological characteristics of a plant, such as organ dimensions and growth rates, (Barnes and Beard 1992) were taken on plants in the pots at bi-weekly intervals. Measurements included plant height (distance from the soil surface to the ligule of the youngest fully expanded leaf), stem diameter (maximum and minimum) at the base of the plant, mature leaf number, length and maximum width of youngest mature leaf. Using these measurements stem volume was calculated by

$$\text{Volume } (cm^3) = \Pi * r_{min} * r_{max} * h$$

Where Π = pi, r_{min} =0.5*stem diameter minimum, r_{max} =0.5*stem diameter maximum, h= stem height. The plants were harvested at ten leaf developmental stage (V10). At harvest, final mass (stem leaf and root) was recorded. Root mass was determined by the following procedure. The pots were dried and the dried soil medium was then laid in a concrete floor: a fine spray of high pressure water was used to separate the roots from the soils. Once the roots are separated they were dried and further peat attached to the soil were removed. Finally the roots were dried at 50 ^{o}C for minimum of four days and the weights were taken. Leaf area of mature, non-senescent leaves was determined using a leaf area meter (CI-203, CID. Inc, Camas, WA), and leaf mass was measured using an analytic balance. Plant biomass and cumulative water use (CWU) was measured at harvest. CWU was calculated from water addition and depletion from pot by taking the difference in weight after watering on a day and measurement before watering during next watering time. Once biomass and water use is calculated TE was determined by

$$TE = \frac{\text{Total biomass of the plant in the pot (g)}}{\text{Total water use by the plant in the pot (kg)}}$$

Photosynthesis and assimilation

Leaf measurements of photosynthesis and assimilation were made using a portable photosynthesis system (LI-6400 portable photosynthesis system, Li-Cor Inc., Lincoln, NE) at vegetative 6th leaf stage. Performance measures of photosynthesis and transpiration included assimilation (A), stomatal conductance (g_s), leaf internal CO_2 partial pressure (C_i), transpiration (T), fraction of photosystem II (PSII) reaction centers which were open (Fv/Fm), and the ratio of electrons, provided by PSII per CO_2 molecule fixed ($Ø_{PSII}/Ø_{CO2}$). Standard measurement

conditions included CO_2 partial pressure (C_a) at 370 µmol mol^{-1}, temperature of 30 ^{o}C, photosynthetic photon flux density of 1200 µmol m^{-2} s^{-1}, and vapor pressure deficit (vpd) adjustments range from 2-4 kPa. Fluorescence was simultaneously measured using a LI-COR LI-6400-40 fluorescence attachment using the manufacture's instructions (revised June 2007). Fluorescence measurements were calibrated to a typical leaf by adjusting the light source to a square flash, adjusting the phase timing to 500, 300, 200 ms interval with 30 % declining ramp during intermediate intervals. The H_2O reference should be around 10kPa at 30°C and the CO_2 reference should stabilize within 3 umol/mol. To have an accurate reading with the instrument, the leaf in the cuvette must attain a steady state. Steady state was observed by viewing a graph of fluorescence and photosynthesis, which showed a steady straight line for close to 2 minutes. Vapor pressure deficit (vpd) was manually controlled for sequential measurements (see below). Measurements were made on the youngest, fully expanded (mature) leaf (as indicated by ligule formation) at the sixth leaf developmental stage (V6), after the leaf had adjusted to cuvette conditions, which typically required 15 minutes. The measurements were taken between 9:30 a.m. and 2:00 p.m. CDT under near full radiation.

Vapor pressure deficit responses

Leaf adjustment to vpd was evaluated by repeating photosynthesis and assimilation measurements for three vpd conditions (1.8, 2.5, or 3.2 kPa). A series of measurements on a given leaf consisted of either ascending or descending vpd conditions. Generally, the sequence alternated for successive leaves.

Dark respiration

The plants (seventh to eighth leaf stage) in the field-pots were used to measure respiration under dark conditions (R_d). Plants were taken into a dark room to allow the plants to acclimate to dark conditions for 2 hours at approximately 25 ^{o}C. Photosynthetic photon flux density (PPFD) in the cuvette was set to zero, and the temperature of the cuvette was set at 30 ^{o}C The youngest mature leaf was selected and using the LI-COR LI-6400 portable photosynthesis system, and measurement of CO_2 efflux from the leaf (R_d) was recorded. After securing the leaf in the cuvette, dark respiration (R_d = - A), stomatal conductance, and internal carbon dioxide

concentration were measured after 2 to 3 minutes. Three measurements were taken at 30 s intervals for every leaf and then averaged.

Leaf angle and orientation

Leaf angle measurements were taken on the plants adjacent to the field-lysimeters. Leaf orientation of the youngest mature leaf was determined by noting the direction that the leaf pointed (N, NW, W, SW, S, SE, E, NE). Leaf angle was measured using a protractor and suspended rod. The angle between the stem (taken as vertical) and the initial slope of the youngest mature leaf blade was measured.

Statistical analysis

Data from the field lysimeter study were analyzed using PROC GLM (SAS-Statistical Analysis Systems v9.13, Cary, North Carolina); using a randomized complete block experimental design. To determine the means separation at 0.05 probability level, Duncan's new multiple range test was used. Tables report adjusted least square means for linear covariate effects (vpd for gas exchange measurements) and for effects of missing values.

Field studies

Crop water use, growth, development and physiological measurements were completed for selected eight genotype of sorghum lines (Table 2.1) grown under field conditions at two locations: the Northwest Research-Extension Center of Kansas State University at Colby, Kansas (39°23′32″N 101°2′51″W; elevation 962m^2) and the Western Kansas Agricultural Research Center of Kansas State University in Hays, Kansas (38°52′46″N 99°19′20″W;elevation 616m).

Experiment and treatment design

The soil type at Colby was a Keith silt loam, buried soil phase, fine-silty, mixed, superactive, mesic aridic argiustolls and at Hays the soil type was a Roxbury fine-silty, mixed, superactive, mesic cumulic haplustolls. Seeds were sown on 9 June 2006 at Hays and on 20 June 2007 at Colby. The plants were grown in four replicated plots (1.4 m x 5.6 m) with a randomized complete block design at Colby and a completely randomized design within irrigation treatment at Hays.Both locations had two irrigation treatments (irrigated and non-

irrigated). The plots were irrigated by flood at Colby and by furrow at Hays. At Hays, three irrigations (37, 56, and 70 days after planting) were applied with 38 mm given at each application. At Colby, two irrigation applications (22 and 77 days after planting) were applied with 78 mm given at each application

Growth and development

At Hays five plants from each plot were identified for allometric measurements and, recorded at bi-weekly intervals. Similarly, at Colby three plants from each plot were selected. Every two weeks, starting from the sixth leaf stage, allometric measurements were taken for each selected plant, including plant height (from soil to ligule of the youngest mature leaf, indicated by ligule formation), stem diameter at the base of the stem (maximum and minimum diameter), length and maximum width of the youngest mature leaf, and mature leaf number. A marker was placed around the base of the sixth leaf to facilitate determination of mature leaf number. After a plant reached the flag-leaf stage, reproductive stages were recorded (Vanderlip, 1972) using the numerical scale of 3=growing point differentiation 4=final leaf visible 5=boot 6=half-bloom 7=soft dough 8=hard dough and 9=physiological maturity. During each measurement period, similar observations were recorded for a representative plant in each plot which was destructively harvested, dried and weighed for above ground biomass determination.

Cumulative water use

Both in Hays and Colby, soil water measurements were taken bi-weekly from the V6 stage through maturity. A neutron probe was used to measure the soil water content. At Hays, soil was excavated by hydraulic-driven tube (38 mm diameter) in each plot to a depth of 1.35 m; a vertical aluminum tube (38 mm diameter and 1.50 m length) was placed inside the hole to provide an access tube. Volumetric water content was determined by the neutron attenuation method (503 DR Hydroprobe, CPN Corp., Martinez, CA), at 0.15 m depth intervals from 0.15 m below the surface to 1.20 m depth. Soil water content was measured in Colby using the same methods, however, the length of the vertical hole was increased to 3.45 m and the counts were measured at 0.30 m intervals to the 3.00 m depth. Access tubes (3.65 m length) were installed in three replicate plots for six of the eight lines where crop stand was considered adequate to represent root water uptake. Wheat straw was placed on the soil at fifth to sixth leaf stage on

plots having neutron-probe access tubes, in order to suppress soil evaporation, at the Colby site in 2007. Precipitation was observed at a National Weather Service observation site located within 1 km of each experimental site.

Soil water depletion was calculated by subtracting the value of stored soil water (calculated from soil water determined by neutron probe at different depths) of one set of measurements from the previous set of measurements for a given plot. Crop water use during bi-weekly intervals was calculated by the sum of soil water depletion, irrigation, and precipitation during the time interval. Cumulative water use was calculated as the sum of bi-weekly crop water use in successive time intervals throughout the growing season.

Photosynthesis and assimilation

Leaf gas exchange measurement was carried out in the field with representative plants at 6^{th} ($V6_F$) and 10^{th} ($V10_F$) leaf stages, using the procedures described in the field lysimeter study used in the green house studies. At the $V6_F$ stage, leaf gas exchange measurements were taken both in irrigated and non-irrigated plots, but for the $V10_F$ stage only irrigated plots were selected. Measurements were conducted on a single representative plant, selected from each plot.

Biomass

The plants identified for allometric measurements were harvested at physiological maturity (indicated by black layer in the grain) for biomass determination. Even late flowering plants were harvested despite incomplete reproductive development. These plants were cut at the base of the stem and dried (minimum of 48 h at 85 ^{o}C) for biomass determination. The panicle of each culm was weighed separately and seed threshed to determine grain weight. At Colby, plant number, culm number, above-ground plant biomass, panicle mass, seed mass and 100 seed mass were also determined for plants in one meter row, at harvest.

Statistical analysis

The data were analyzed using PROC GLM (SAS-Statistical Analysis Systems v9.13, Cary, North Carolina). In Hays data, a completely randomized design was used whereas for Colby randomized a complete block design model was used. A split plot treatment design was used at both sites with irrigation as whole plot and lines as split plot effects. To determine the means separation at 0.05 probability level, Duncan's new multiple range test was used. Tables

report least square means, adjusted for linear covariate effects (vpd for physiological measurements) and effects of missing values.

Results

Greenhouse study (Manhattan)

Plants with high transpiration efficiency (TE) emerged better than plants with low transpiration efficiency (Fig. 1). By Day 16, an average of four plants with high TE had emerged and an average of three plants with low TE lines had emerged. Even though six germinated seeds were planted in each pot, no pot of any of the eight lines had six seedlings emerge.

Under both well-watered and drought-stressed conditions, height of the low TE plants was similar to that of the high TE plants (Table 2.2). Drought did not affect the height of the low TE plants. However, at harvest, the high TE plants were taller under well-watered conditions than the high TE plants under drought-stressed conditions.

Under both watering regimes, the pressure potential of the high TE plants at harvest did not differ from that of low TE plants (Table 2.3). The pressure potential of the well-watered, low TE plants was similar to that of the drought-stressed, low TE plants. However, the pressure potential of the well-watered, high TE plants was higher than that of the drought-stressed, high TE plants.

Relative water content of plants with high TE lines did not differ from that of plants with low TE (Table 2.4). Because of the large variability in measurements, the relative water content of well-watered plants did not differ from that of drought-stressed plants. As plants aged, relative water content decreased.

When all dates were averaged together, the average stomatal resistances ($\pm$ standard deviations) of low TE lines under well-watered and dry conditions were 1960 ± 372 and 1733 ± 452 s/m, respectively (Table 2.5). For the high TE lines, these values were 2070 ± 537 and $2593\pm$ 448 s/m, respectively. There was great variation in stomatal resistance values, and many of the values were high. Values over about 2000 s/m meant that the stomata were essentially closed. However, the data did indicate that the low and high TE lines had similar stomatal resistances under well-watered conditions and that, under drought, the low TE lines kept their stomata more open than did the high TE lines.

The water used by low TE plants was similar to that of high TE plants (Table 2.6). Plants that were drought-stressed used about 60% of the water that was used by the plants that were well watered (i.e., 778 mL/1411 mL ≈ 0.60; 825 mL/1360 mL ≈ 0.60).

At harvest, the number of low TE plants surviving was similar to that of high TE plants. Fresh and dry shoot weights of low TE plants were similar to those of high TE plants. Also, the root weights of low TE plants were similar to those of high TE plants. Drought did not affect the biomass of the low TE plants. However, the fresh shoot weight of well-watered, high TE plants was greater than that of drought-stressed, high TE plants (Table 2.7, last row; 6.8 versus 3.9 grams per pot).

Field lysimeter and field study (Colby and Hays)

At Hays, there was nearly 85- 90% stand in each plot; all eight lines were measured for water balance. In Colby 2007, for water balance measurement, plots only with 80-100% population were identified, so we ended up with three replications and six lines for the soil water balance. Results from the Colby 2006 field trial are not included due to inadequate stand establishment. There was poor germination; stand establishment was inadequate in most plots and heterogeneous plant phenotypes appeared to compromise stand integrity.

Lines differed in all growth and development parameters observed at Hays (2006, Table 2.8). Likewise, groups of low TE and high TE lines differed in all parameters but tiller number and internode length, Lines PI586381 and PI267532 were slow to reach reproductive stage (Table 2.9); line PI 267532 had greatest leaf number while line PI 586381 had greatest stem length and stem volume. Line PI295121 had most tillers. Irrigation altered responses of lines for the total number of mature leaves, stem length and volume. No differences in biomass were detected in Colby (2007) field studies.

Lines differed in biomass and all biomass fractions at Hays (2006, Table 2.10). The high TE lines had greater leaf fractions and less stem fractions than did low TE lines (Table 2.11). Irrigation increased cumulative water use; however there were no differences in cumulative water use among (Table 2.11). Lines PI533946 and PI584085 had greatest leaf fractions. Lines PI586381 and PI267532 had greatest stem fraction. Lines PI567939, PI295121 and PI91652 had greatest grain fraction. Line PI586381 had greatest biomass. Lines for which reproductive development was delayed (PI586381 and PI267532) also had greatest stem fraction and no grain

formation. These growth measurements indicate substantial differences in growth and development among these lines.

Analysis of variance for physiological parameters for field-grown plants during the sixth leaf stage (Colby, 2007) is presented in Table 2.12. Effects of line interacted with irrigation effects for assimilation, transpiration, fluorescence and quantum yield ratio. All variables except quantum yield ratio had greater values under irrigated conditions. Smaller values of quantum yield ratio correspond with more efficient use of light for assimilation. PI295121 and PI533946 showed significant difference with irrigated and non-irrigated condition among variables, which could account for the difference in the interaction. Low TE lines had greater A, T, g_s, C_i Fv/Fm and lower quantum yield ratios than high TE lines under irrigated conditions. For non-irrigated conditions, low TE lines had greater C_i than high TE lines. Vapor pressure deficit strongly altered T, with significant effects on A and C_i. There were also differences in assimilation, transpiration, stomatal conductance, internal carbon dioxide, fluorescence and quantum use efficiency among lines. Lines PI295121 and PI533946 exhibited generally the greatest assimilation, transpiration, fluorescence and internal carbon dioxide and least quantum yield ratio (Table 2.13). Quantum yield ratio was greatest for line PI586381 under non-irrigated condition,

The analysis of variance for physiological parameters for field-grown plants during the tenth leaf stage (Colby, 2007, irrigated condition) is presented in Table 2.14. Assimilation, internal carbon dioxide and fluorescence differed among lines. Vapor pressure deficit affected transpiration. Lines PI295121, PPI586381 and PI533946 exhibited greatest assimilation and fluorescence (Table 2.15). Line PI257309 exhibited greatest internal carbon dioxide. No differences in physiological parameters were detected between low TE and high TE groups of lines; no differences among lines were detected for transpiration and quantum yield ratio.

The analysis of variance for physiological parameters for field-grown plants in lysimeters during the sixth leaf stage (Colby, 2007) is presented in Table 2.16 Assimilation, quantum yield ratios and fluorescence differed among lines; vapor pressure deficit altered transpiration. High TE lines, as a group, exhibited greater fluorescence than low TE lines, as a group. Lines PI267532 and PI533946 exhibited greatest assimilation, fluorescence and quantum yield ratio (Table 2.17). No differences in transpiration, stomatal conductance and internal carbon dioxide were detected among lines.

Considering physiological measurements among all three sets of observations, some consistent differences were observed. Assimilation, stomatal conductance and fluorescence differed among lines in all three studies. Lines PI295121 and PI533946 exhibited generally greatest assimilation, transpiration, stomatal conductance and fluorescence, and least quantum yield ratio. Line PI533946 generally exhibited lower C_i than PI295121. Consistent differences among TE groups observed at sixth leaf stage under field irrigated conditions were not repeated at the tenth leaf stage nor for field lysimeters, at the sixth leaf stage.

Lines differed in water use in the field lysimeter study at Colby (2007, Table 2.18) with line PI586381 exhibiting greatest transpiration. No differences were detected in biomass nor TE was detected among lines or between the TE groups. Lines differed in internal carbon dioxide; line PI533946 exhibiting greatest C_i and numerically least stomatal conductance and leaf respiration (Table 2.19). However, no differences among lines were detected for respiration, conductance or leaf angle. Irrigation increased cumulative water use at Colby (Table 2.20) and high TE lines used more water in non-irrigated conditions (Table 2.21). There was no difference in the biomass taken for one meter in the row during final harvest at Colby, 2007 (Table 2.22, 2.23)

Figure 2.1 Emergence rate of both high and low transpiration lines for initial few days after planting

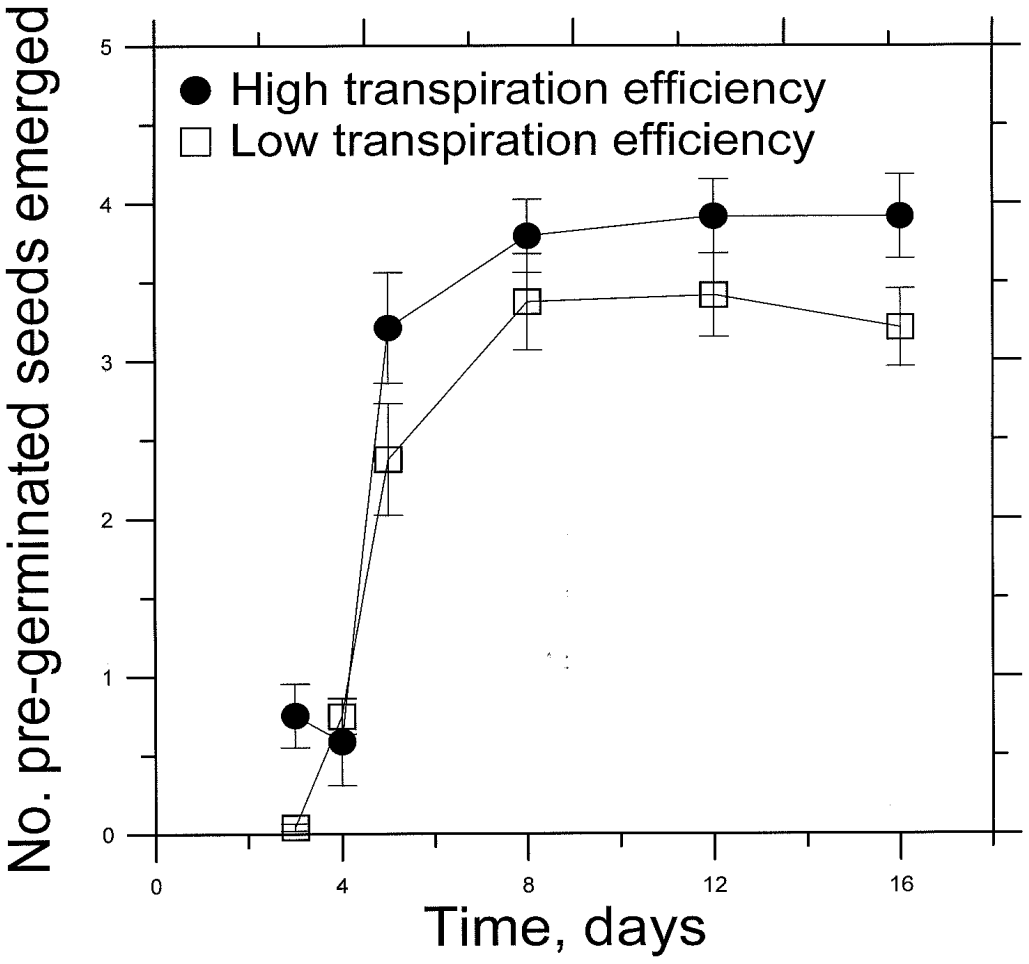

Table 2.1 Grain sorghum lines varying in transpiration efficiency used in the experiments.

Plant I.D	Origin	Race	TE
PI257309	**Argentina**	*Guinea-bicolor*	**Low**
PI295121	**Australia**	*Caudatum*	**Low**
PI586381	**Cameroon**	*Guinea-caudatum*	**Low**
PI267532	**India**	*Kafir-caudatum*	**Low**
PI567933	**China**	*Bicolor*	**High**
PI391652	**China**	race unknown	**High**
PI533946	**India**	*Durra-bicolor*	**High**
PI584085	**Uganda**	*Caudatum*	**High**

Table 2.2 Height (cm) of four lines of sorghum with low transpiration efficiency (TE) and four lines with high TE at two different times during the experiment. Day 0 was the day that seed germination began. Un-watered plants received water for the first 28 days. Harvest was Day 75. Mean and standard deviation are shown, All plants in a pot were measured for height (number of plants in a pot varied between zero to three plants), and there were three pots treatment.

	Height, 46 d		Height, 75 d	
	Watered	Un-watered	Watered	Un-watered
Low TE				
PI257309	25.9±6.2	20.1±2.2	32.0±3.58	8.3±14.4
PI295121	†....	5.7±9.8	9.8±17.0	12.8±22.2
PI586381	11.8±10.4	22.7±3.3	13.0±12.3	18.5±16.7
PI267532	28.7±1.6	11.5±20.0	44.0±6.5	5.7±9.8
Aver.	16.6±13.3	15.0±7.8	24.7±16.2	11.3±5.6
High TE				
PI567933	28.5±12.3	35.8±8.5	44.3±15.2	32.8±29.5
PI391652	19.3±20.6	20.9±6.5	36.3±51.3	17.7±15.5
PI533946	11.8±11.1	15.6±13.5	28.2±24.8	23.8±20.7
PI584085	33.8±3.9	20.8±5.6	43.7±16.0	18.7±16.4
Aver.	23.4±9.8	23.2±8.7	38.1±7.5	23.3±6.9

†.... No plants in any of the three pots.

Table 2.3 Pressure potential (bars) of four lines of sorghum with low transpiration efficiency (TE) and four lines with high TE at the end of the experiment (75 days after the start of germination). Mean and standard deviation are shown. The number of plants used in the average is shown in parenthesis after the mean and standard deviation

	Pressure potential	
	Watered	Un-watered
Low TE		
PI257309	-17.2±3.5 (3)	-17.3±11.9 (3)
PI295121	-6.5 (1)	-12.5 (1)
PI586381	-25.5±0.7 (2)	-23.5±3.5 (2)
PI267532	-11.5±11.8 (3)	-22.3±15.2 (2)
Aver.	-15.2±8.2	-18.9±5.0
High TE		
PI567933	-6.3±3.9 (3)	-22.2±5.5 (3)
PI391652	-17.3±7.4 (2)	-20.0±9.5 (3)
PI533946	-9.3±3.2 (2)	-23 (1)
PI584085	-11.2±2.0 (3)	-19.0±3.5 (3)
Aver.	-11.0±4.6	-21.1±1.9

Table 2.4 Relative water content of four lines of sorghum with low transpiration efficiency (TE) and four lines with high TE on three different dates during the experiment. Plants were grown under well-watered or drought-stressed conditions. Day 0 was the day that seed germination began and harvest was Day 75. Mean and standard deviations are shown. Values are the average of three measurements, except where shown in parenthesis, when they are single values or the average of two values. Average values of the four low TE lines and four high TE lines under wet and dry treatments are shown in the right two columns.

	Low transpiration efficiency									
	PI257309		PI295121		PI586381		PI267532		Watered	Un-watered
Day	Watered	Un-watered	Watered	Un-watered	Watered	Un-watered	Watered	Un-watered	Ave.	Ave.
1	0.929±0.012	0.898±0.030	†....	0.943 (1)	0.888 (1)	0.920±0.002	0.928±0.018	0.921 (1)	0.933± 0.008	0.920± 0.018
1	0.855±0.0(2)	0.783±0.159	0.904 (1)	0.938 (1)	†....	0.838±0.077	0.885±0.054	0.673 (1)	0.838± 0.057	0.808± 0.110
5	0.678±0.187	0.566±0.314	0.753 (1)	0.879 (1)	0.700+0.081(2)	0.692 (1)	0.689±0.266	0.541±0.392 (2)	0.705± 0.033	0.672± 0.152
	High transpiration efficiency									
	PI567933		PI391652		PI533946		PI384085		Watered	Un-watered
	Watered	Un-watered	Watered	Un-watered	Watered	Un-watered	Watered	Un-watered	Ave.	Ave.
1	0.912±0.017	0.894±0.024	0.943±0.0(2)	0.898±0.053	0.947±0.01(2)	0.901±0.015	0.918±0.040	0.901±0.012(2)	.932± .06	0.899± 0.003
1	0.828±0.091	0.844±0.092	0.875±0.0(2)	0.841±0.110	0.919±0.00(2)	0.783±0.112	0.888±0.031	0.825±0.015(2)	.878± .038	0.823± 0.028
5	0.786±0.156	0.596±0.350	0.794±0.0(2)	0.611±0.227	0.831±0.06(2)	0.673±0.13(2)	0.557 (1)	0.575±0.323(2)	.742± .125	0.614± 0.042

†... No plants

Table 2.5 Stomatal resistance (s/m) of four lines of sorghum with low transpiration efficiency (TE) and four lines with high TE on 10 different dates during the experiment. Plants were grown under well-watered or drought-stressed conditions. Day 0 was the day that seed germination began. Harvest was Day 75. Mean and standard deviations are shown. Values are the average of the number of measurements shown in parenthesis. Values averaged over the 10 dates are shown at the bottom of columns.

	Low transpiration efficiency							
	PI257309		PI295121		PI586381		PI267532	
Day	Watered	Un-watered	Watered	Un-watered	Watered	Un-watered	Watered	Un-watered
31	2237±1975 (5)	2425±1077 (3)	9578 (1)	†....	1505 (1)	1716±898 (4)	4590±1029 (3)	2614 (1)
33	2661±1849 (5)	2108±272 (6)	1404 (1)	†....	744 (1)	876±428 (3)	3341±2975 (3)	1617 (1)
35	1722±192 (6)	3383±2968 (7)	3287 (1)	1547 (1)	521 (1)	1031±707 (3)	1280+710 (4)	520 (1)
37	999±319 (7)	1523±230 (7)	980 (1)	999 (1)	565±231 (2)	1264±325 (3)	1328±1080 (4)	835 (1)
39	2252±2301 (7)	1959±1718 (7)	1183 (1)	1017 (1)	552±107 (2)	1618±1934 (4)	3272±3212 (4)	426 (1)
41	1662±338 (6)	3258±889 (7)	2289 (1)	†....	1304±539 (2)	3427±1636 (4)	1972±561 (4)	2969 (1)
43	1960±2992 (7)	1846±796 (7)	1032 (1)	1360 (1)	1733±1310 (6)	1963±471 (3)	1390±558 (4)	613 (1)
46	1487±806 (8)	2670±2874 (7)	†....	1046 (1)	631±170 (2)	2154±1765 (4)	955±260 (3)	2027 (1)
48	1535±870 (8)	1848±1222 (7)	1177 (1)	1307 (1)	2248±1959 (4)	1999±299 (4)	1413±828 (4)	5523 (1)
50	956±639 (6)	1087±383 (7)	741 (1)	613 (1)	991±28 (2)	1273±572 (4)	1517±465 (5)	1468 (1)
Ave	1747±56	2211+730	2408+2805	1127+306	1577±1498	1732±734	2106±1203	1861±1560
	High transpiration efficiency							
	PI567933		PI391652		PI533946		PI584085	
	Watered	Un-watered	Watered	Un-watered	Watered	Un-watered	Watered	Un-watered
31	4984±3283 (7)	5489±3226 (3)	784 (1)	3616±2544 (7)	1803±440 (2)	4546±5657 (5)	2795±1838 (6)	8538±3967 (6)
33	3897±2441 (5)	8840±8689 (4)	1221±118(2)	2724±1920 (7)	2879 (1)	2716±+2115(5)	2159±1137 (7)	2745±2343 (4)
35	1847±1064 (6)	1432±830 (4)	1568±105(2)	1775±1445 (5)	1470 (1)	2224±2692 (4)	977±528 (7)	1413±837 (7)

37	985±202 (5)	1166±299 (4)	756±302 (2)	1408±522 (7)	994±478 (2)	1998±1452 (5)	1355±607 (7)	1190±331 (5)
39	2112±1315 (6)	1982±1826 (4)	998±419 (2)	2157±2046 (7)	4783±3773 (2)	1133±577 (5)	1327±933 (6)	941±488 (7)
41	3836±1510 (6)	4712±1078 (4)	2542±112(2)	3245±930 (7)	4211±2079 (4)	2396±1554 (5)	4988±2338 (6)	3967±3352 (4)
43	2554±2204 (4)	1783±816 (5)	1143±294(2)	2076±785 (6)	2653±1225 (3)	2411±1720 (5)	1293±697 (7)	1605±612 (7)
46	1999±1149 (7)	2344±1717 (4)	1409±242(2)	2224±1600 (6)	1449±1151 (3)	2809±1842 (5)	1302±548 (7)	1164±1084 (7)
48	1294±728 (4)	2701±759 (4)	2025±686(2)	2346±453 (6)	2076±907 (3)	1850±582 (5)	1506±297 (5)	2286±1122 (7)
50	1114±578 (6)	2062±1937 (6)	1549±271(2)	1433±617 (6)	1406±504 (3)	1099±368 (5)	1259±1068 (6)	1194±439 (7)
Ave	2612±1340	3251±2412	1400±557	2300±723	2372±1267	2318±977	1896±1210	2504±2317

†.... No plants.

Table 2.6 Water use (mL) of four lines of sorghum with low transpiration efficiency (TE) and four lines with high TE during the experiment. All pots were watered to pot capacity at the beginning of the experiment. After that, each wet-treatment pot and each dry-treatment pot received about 700 mL and about 300 mL water, respectively, during the experiment. Mean and standard deviation are shown (n = 3 pots per treatment)

	Water use	
	Watered	Un-watered
Low TE		
PI257309	1797±106	915±84
PI295121	1030±476	719±266
PI586381	1215±430	848±159
PI267532	1602±257	629±107
Aver.	1411±351	778±128
High TE		
PI567933	1548±166	853±121
PI391652	1185±329	813±236
PI533946	1140±361	802±70
PI584085	1566±33	831±120
Aver.	1360±229	825±22

Table 2.7 Fresh and dry weights (grams) at harvest of low and high transpiration efficiency lines of sorghum grown under well-watered and dry conditions

	Low		High	
	Wet	**Dry**	**Wet**	**Dry**
Fresh wt.	4.0±3.6	1.8±1.1	6.8±0.9	3.9±1.2
Dry wt.	1.3±1.2	0.7±0.5	2.1±0.8	1.5±0.4

Table 2.8 Analyses of variance for allometric data studied in high and low transpiration efficient sorghum lines grown under dry land and irrigated conditions at Hays, KS in 2006.

Type III Mean Squares

Source of Variation	df	Reproductive stage		Total matured leaf	Tiller number	Stem length	Stem volume	Internode length	Flag length
		85 DAP	119 DAP						
Irr	1	1.47	0.510	3.10	0.560	204.9	197586.4	0.031	3803.4
Error a	2	0.717	2.93	213.2	0.300	619.2	262655.5	0.945	1102.1
Line	7	5.94***	15.33***	804.4***	1.80**	17425.5***	1741882.0***	81.97***	76766.0***
Irr*line	7	0.344	0.607	215.09*	0.189	737.58*	150187.6*	1.62	3507.7
Error B	70	0.527	0.418	62.93	0.454	228.7	62182.9	1.30	3992.9
Low TE vs High TE	1	9.95***	10.57***	754.4**	0.207	17137.9***	4153977.4***	4.0	198040.2***

*, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.9 Mean value of allometric data studied in high and low transpiration efficient sorghum lines studied under irrigated and dry land conditions at Hays, KS in 2006

Least Square Mean

Line	Reproductive stage		Total matured leaf		Tiller number (cm)	Stem length (cm)		Stem volume (cm)		Internode length (cm)	Flag leaf length (cm)
	85 DAP	119 DAP	Irr	Dry		Irr	Dry	Irr	Dry		
Low TE											
PI 257309	6.0bc	9.0a†	15.75c	15.58d	0.25ab	178.50ba	163.71a	437.3bc	318.73dc	11.34a	33.50e
PI 295121	6.66bc	9.0a	15.66c	16.33d	1.33a	65.33d	72.33d	246.7c	251.61dc	4.17c	48.66b
PI 586381	5.50dc	6.0c	21.66b	21.40b	.00b	192.50ba	157.40ab	1853.5a	1121.91a	8.82b	68.83a
PI 267532	5.0d	6.10c	24.80a	26.17a	.40ab	135.80ba	94.50c	991.2b	700.0b	5.47c	32.80ed
Mean	5.79	7.52	19.46	19.98	0.49	143.03	121.99	882.17	598.07	7.45	45.94
High TE											
PI 567933	6.71ab	9.0a	15.75c	14.88d	.00b	165.50ba	145.83b	285.3c	240.80dc	11.39a	36.14cd
PI 391652	7.40a	9.0a	15.20c	14.67d	1.0ab	110.40c	108.67c	82.2c	139.60d	7.30b	24.80ed
PI 533946	6.75ab	9.0a	15.00c	16d	1.0ab	65.00d	65.10d	165.9c	227.65dc	4.23c	23.50e
PI 584085	6.0bc	7.0b	16.50c	18.67c	.00b	67.50d	73.50d	291.8c	409.74c	4.09c	47.00cb
Mean	6.71	8.5	15.61	16.05	0.50	102.1	98.28	206.3	254.44	6.75	32.86
Critical value	0.857	0.153	3.14	2.32	0.993	31.82	14.75	616.7	189.3	1.64	11.41
Low TE ns vs High TE	ns	ns	*	*	ns	***	***	ns	ns	ns	***

†Least squares means followed by the same letter do not differ at the 0.05 level of probability, DMRT

††Linear contrast testing against null effects of low TE and high TE lines; *, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.10 Analyses of variance for final harvest data studied in high and low transpiration efficient sorghum lines grown under dry land and irrigated conditions at Hays, KS in 2006.

		Type III Mean Squares				
Source of Variation	**df**	**Fraction stem**	**Fraction leaf** $10x^{-3}$	**Fraction grain**	**Biomass**	**CWU**
Irr	1	0.006	7.67	0.011	1589.7	45.94***
Error a	2	0.033	270.18	0.040	2382.1	2.04
Line	7	0.145***	4838.9***	0.173***	11040.7*	0.831
Irr*line	7	0.020	1302.8***	0.020	28784.9***	0.827
Error B	70	0.014	224.0	0.017	3809.7.0	1.92
Low TE vs High TE	1	0.167**	1858.5*	0.151	8766.0	0.002

*, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.11 Mean value of final harvest data studied in high and low transpiration efficient sorghum lines studied under irrigated and dry land conditions at Hays, KS, 2006.

	Least Square Mean							
Lines	**Fraction Leaf (g)**		**Fraction stem (g)**	**Fraction grain (g)**	**Biomass (g)**		**CWU**	
	Irr	**Dry**			**Irr**	**Dry**	**Irr**	**Dry**
Low TE								
PI 257309	0.13bc†	0.14e	0.60ab	.21ab	89 b	118.08ab	25.8a	13.74a
PI 295121	0.21b	0.24c	0.40ab	.30ab	67 b	142.37ab	24.9a	15.71a
PI 586381	0.16bc	0.19d	0.78a	.00b	474a	70.03b	24.8a	14.64a
PI 267532	0.22b	0.25c	0.74a	.00b	162 b	144.65ab	24.8a	14.54a
Mean	0.18	0.21	0.63	0.127	159.75	118.78	20.75	15.65
High TE								
PI 567933	.09c	0.12e	0.55ab	.28ab	148 b	188.91a	24.0 a	14.93
PI 391652	0.16bc	0.18de	0.42ab	.32a	148 b	98.51ab	24.4 a	15.23a
PI 533946	0.39a	0.36b	0.60ab	.00b	21 b	150.14ab	24.8a	15.17a
PI 584085	0.39a	0.44a	0.60ab	.00b	53b	148.02ab	25.4a	15.20a
Mean	0.25	0.28	0.54	0.15	122.25	146.40	19.9	15.13
Critical value	0.098	0.045	0.259	0.273	134.3	91.8	2.8	2.12
†† Low TE Vs High TE	***	***	**	ns	ns	ns	ns	ns

†Least squares means followed by the same letter do not differ at the 0.05 level of probability, DMRT
††Linear contrast testing against null effects of low TE and high TE lines; *, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.12 Analyses of variance for physiological traits studied in high and low transpiration efficient sorghum lines evaluated at V6 stage under irrigated and non-irrigated field conditions at Colby, KS 2007. Analyses were conducted using vapor pressure deficit as a covariate in the model.

Type III Mean Squares

Source of variation	df	A	T	g_s x 10^{-3}	C_i	Fv/Fm x 10^{-3}	$Ø_{PSII}$: $Ø_{CO2}$
Rep	3	100.4	3.956	4.93	5764.6	9.293	1.483
Irr	1	584.5**	37.925**	46.16**	3529.6*	18.002*	16.615**
Error a	3	211.0	8.191	11.58	2131.6	9.506	4.302
Line	5	237.6***	18.849***	23.79***	3444.0***	11.941***	2.561*
Irr*Line	5	119.2*	7.909*	6.79	1287.3	5.229*	3.206*
Error b	81	41.2	3.331	3.48	661.6	1.922	1.114
Vpd	1	266.6*	88.385***	3.75	2938.8*	7.287	3.233
Low TE vs High TE	1	350.3**	45.385***	58.78***	10686.9***	24.863***	4.524

*, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.13 Mean value of physiological traits studied in high and low transpiration efficient sorghum lines evaluated at V6 stage under irrigated and non-irrigated field conditions at Colby, KS, 2007.

Least Square Means

	A $\mu mol\ m^2\ s^{-1}$		**T** $mmol\ m^2\ s^{-1}$		**g^s** $mol\ m^2\ s^{-1}$		**C_i** $\mu mol\ mol^{-1}$		**Fv/Fm**		**$Ø_{PSII}$: $Ø_{CO2}$** $mol^{e-}\ mol\ CO_2^{-1}$	
	Irri	dry	Irri	dry	Irri	dry	Irri	dry	Irri	dry	Irri	dry
Low TE												
PI295121	47.0a†	34.2a	10.50a	6.12a	0.348a	0.219a	107.6a	69.3ab	0.603a	0.518a	9.70c	10.87b
PI586381	38.7b	31.1a	7.78bc	6.48a	0.253b	0.200a	88.1ab	83.9a	0.558a	0.530a	10.30bc	12.69a
Mean††	42.8	32.7	9.14	6.30	0.300	0.210	97.9	76.6	0.580	0.524	10.0	11.78
High TE												
PI567933	35.4b	28.8a	6.64cd	4.89a	0.213bc	0.154a	64.4bc	39.7c	0.528b	0.484a	10.64ab	11.46ab
PI391652	31.5b	27.4a	5.25cd	5.12a	0.182cd	0.161a	57.7c	66.5bc	0.499bc	0.493a	11.05a	11.48ab
PI533946	44.2a	35.5a	8.73b	6.59a	0.281b	0.216a	73.8bc	76.9ab	0.588a	0.530a	9.75c	11.20b
PI584085	27.8c	33.5a	5.24cd	5.64a	0.163d	0.182a	72.2bc	41.6bc	0.474c	0.506a	11.14a	10.65b
Mean††	34.7	30.8	6.47	5.64	0.210	0.178	66.5	55.6	0.522	0.502	10.65	11.20
Critical Value†	4.9	8.3	1.60	2.21	0.051	0.072	28.5	27.3	0.042	0.052	0.69	1.43
†† Low TE Vs High TE	***	ns	***	ns	***	ns	**	*	***	ns	**	ns

†Least squares means within a column, adjusted for linear vpd effects, followed by the same letter do not differ at the 0.05 level of probability, DMRT
††Linear contrast testing against null effects of low TE and high TE lines; *, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.14 Analyses of variance for physiological traits studied in high and low transpiration efficient sorghum lines evaluated at the V10 stage under irrigated and field conditions at Colby, KS, 2007. Analyses were conducted using vapor pressure deficit as a covariate in the model.

		Type III Mean Squares					
Source of Variation	**df**	**A**	**T**	**g_s x 10^{-6}**	**C_i**	**Fv/Fm x 10^{-6}**	**$Ø_{PSII}$: $Ø_{CO2}$**
Rep	3	77.4	0.969	795.58	3800.3	747.10	1.473
Line	7	148.5**	4.721	4421.76	2905.1*	1096.19**	2.981
Error B	26	39.2	2.190	2030.74	1454.3	202.35	1.601
Vpd	1	2.5	24.128*	6.41	1020.8	5.84	0.205
Low TE vs High TE	1	4.36	0.125	233.77	25.8	24.33	0.477

*, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.15 Mean value of physiological traits studied in high and low transpiration efficient sorghum lines evaluated at V10 stage under irrigated and field conditions at Colby, KS, 2007.

Least Square Means

Line	A µmol m^2 s^{-1}	T mmol m^2 s^{-1}	g_s mol m^2 s^{-1}	C_i µmol mol^{-1}	Fv/Fm	$Ø_{PSII}$: $Ø_{CO2}$ mol $^{e-}$ mol CO_2^{-1}
Low TE						
PI 257309	19.5ab†	4.20ab	0.130ab	108.5ab	0.380b	12.84a
PI 295121	27.6a	5.64a	0.176a	86.4b	0.460a	11.91a
PI 586381	25.5a	5.09a	0.157ab	75.2b	0.454a	11.14a
PI 567933	15.6b	2.75ab	0.092b	83.2b	0.377a	11.20a
Mean	22.05	4.42	0.139	88.32	0.418	11.78
High TE						
PI 391652	25.0a	5.05ab	0.155ab	86.1b	0.450b	11.71a
PI 267532	14.6b	3.44b	0.106ab	134.2a	0.346b	12.75a
PI 533946	27.8a	5.10ab	0.162ab	59.3b	0.463a	10.80a
PI 584085	18.0b	3.60ab	0.109abc	80.83b	0.391ab	12.80a
Mean	21.35	4.29	.133	90.09	0.410	12.01
Critical Value	9.0	2.12	0.064	46.0	0.064	1.82
†† Low TE Vs High TE	ns	ns	ns	ns	ns	ns

†Least squares means within a column, adjusted for linear vpd effects, followed by the same letter do not differ at the 0.05 level of probability, DMRT
††Linear contrast testing against null effects of low TE and high TE lines; *, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.16 Analyses of variance for physiological traits studied in high and low transpiration efficient sorghum lines evaluated at V6 stage in a mini lysimeter under irrigated field conditions at Colby, KS, 2007. Analyses were conducted using vapor pressure deficit as a covariate in the model.

		Type III Mean Squares					
Source of variation	**df**	**A**	**T**	**g_s**	**C_i**	**Fv/Fm**	**$Ø_{PSII}$: $Ø_{CO2}$**
Rep	3	116.0	4.876	5.33	180.8	61.580	3.180
Line	7	72.8**	2.800	2.61	351.3	5.96**	4.600*
Error B	24	18.8	1.159	1.18	478.4	1.011	1.539
Vpd	1	5.8	25.467***	0.004	230.6	0.0934	0.023
Low TE vs High TE	1	36.2	0.351	.020	1540.5	112.567**	5.700

*, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.17 Mean value of physiological traits studied in high and low transpiration efficient sorghum lines evaluated at the V6 stage mini lysimeter field conditions at Colby, KS 2007.

Least Square Means

Line	A µmol m^2 s^{-1}	T mmol m^2 s^{-1}	g_s mol m^2 s^{-1}	C_i µmol mol^{-1}	Fv/Fm	$Ø_{PSII}$: $Ø_{CO2}$ mol $^{e-}$ mol CO_2^{-1}
Low TE						
PI 257309	21.8c †	4.37a	0.140b	93.7a	0.424c	12.27a
PI 295121	27.3c	4.69a	0.170ab	79.7a	0.510ab	10.64abcd
PI 586381	25.1c	4.84a	0.155b	82.3a	0.461bc	11.40b
PI 567933	27.2c	5.29a	0.168ab	81.5a	0.494ab	10.45bcd
Mean	25.3	4.8	0.16	84.4	0.472	11.20
High TE						
PI 391652	19.8c	3.75a	0.119b	77.5a	0.463ab	10.88abcd
PI 267532	29.9c	5.53a	0.174ab	63.1a	0.532a	9.59bc
PI 533946	32.9cb	6.45a	0.202a	75.4a	0.539a	9.25d
PI 584085	27.2c	5.29a	0.157ab	65.6a	0.504a	11.67abc
Mean	27.5	5.26	.163	70.4	.510	10.35
Critical Value	6.17	1.53	0.048	31.1	0.045	1.76
†† Low TE Vs High TE	ns	ns	ns	ns	**	ns

†Least squares means within a column, adjusted for linear vpd effects, followed by the same letter do not differ at the 0.05 level of probability, DMRT
††Linear contrast testing against null effects of low TE and high TE lines; *, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.18 Analyses of variance for final harvest and physiological traits studied in high and low transpiration efficient sorghum lines evaluated under field lysimeter studied under irrigated condition at Colby. KS in 2007

Type III Mean Squares

Source of Variation	df	Water use	Total Biomass	TE	Dark Respiration	Dark Conductance	Dark Internal CO_2	Leaf Angle
Rep	1	1258443.2	34.891	7.79	28.78	0.005	1082255.6	.38
Line	7	832454.8**	22.238	1.77	1.18	0.0002	81466.4*	50.01
Error B	19	158287.84	12.461	1.28	0.90	0.00004	22130	35.35
Low TE vs High TE	1	28857.9	13.17	1.95	0.00	0.0002	12984.3	23.70

*, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.19 Mean value of final harvest and physiological traits studied in high and low transpiration efficient sorghum lines evaluated under field lysimeter condition studied under irrigated conditions at Colby. KS in 2007

	Least Square Means						
	Water use g	**Total Biomass** g	**TE** g/kg	**Dark Respiration** µmol m^2 s^{-1}	**Dark Conductance** x 10^{-3} mol m^2 s^{-1}	**Dark Internal CO_2** µmol mol^{-1}	**Leaf Angle** (degree)
Low TE							
PI 257309	1506.6c	4.90 bc†	3.06 a	1.49ab	9.05ab	488.5cb	12.63ab
PI 295121	1891.0bc	7.03 abc	3.55 ab	2.22ab	14.27ab	582.5b	12.33ab
PI 586381	3127.0a	10.90 ab	3.40 ab	1.70ab	24.55ab	536.3cb	21.78a
PI 267532	2031.1bc	7.11 abc	3.13 ab	2.04ab	24.0ab	456.8cb	11.63b
Mean	2158.5	7.48	3.33	1.85	18.0	515.3	14.58
High TE							
PI 567933	1932.5	12.33 a	2.80 b	1.48b	30.6a	311.7c	11.46ab
PI 391652	2193.7bc	4.20 c	5.11 b	3.20ab	10.0ab	589.9b	13.13b
PI 533946	2171.2 bc	8.17 abc	3.57 ab	0.92a	7.8a	882.3a	17.04a
PI 584085	2524.5b	8.97 abc	3.84 ab	2.02ab	13.6ab	457.2cb	9.75b
Mean	2159.5	8.41	3.71	1.9	15.5	460.3	12.84
Critical Value	647.3	3.38	1.84	1.55	0.02	242.9	8.90
†† Low TE Vs High TE	ns	ns	ns	ns	ns	ns	ns

†Least squares means followed by the same letter do not differ at the 0.05 level of probability, DMRT

††Linear contrast testing against null effects of low TE and high TE lines; *, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.20 Analyses of variance for final harvest data studied in high and low transpiration efficient sorghum lines grown under dry land and irrigated conditions at Colby, KS in 2007.

Type III Mean Squares

Source of variation	df	CWU	Biomass
Rep	2	0.948	3278.5
Irr	1	501.984**	222.6
Error A	2	0.436	636.8
Line	5	20.301	2660.2
Irr*Line	5	42.679	183.5
Error B	20	22.758	1403.7
Low TE vs High TE	1	26.673	555.2

*, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.21. Mean value of final harvest data studied in high and low transpiration efficient sorghum lines grown under dry land and irrigated conditions at Colby, KS in 2007.

Least Square Means

	CWU (cm)		Biomass (g/culm^{-1})	
	Irr	Dry	Irr	Dry
Low TE				
PI 295121	45.17a†	28.91b	66.06a	77.59a
PI 586381	45.14a	35.56ab	73.67a	92.98a
Mean	45.15	32.23	69.86	85.28
High TE				
PI 567933	44.37a	40.12a	62.58a	58.94a
PI 391652	42.21a	34.91ab	77.75a	69.32a
PI 533946	44.43a	37.40ab	88.71a	86.88a
PI 584085	40.56a	40.17a	115.07a	128.00a
Mean	42.89	38.15	86.02	85.78
Critical Value	7.63	7.90	70.11	67.09
†† Low TE Vs High TE	ns	*	ns	ns

†Least squares means followed by the same letter do not differ at the 0.05 level of probability, DMRT

††Linear contrast testing against null effects of low TE and high TE lines; *, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.22 Analyses of variance for final one meter row harvest data studied in high and low transpiration efficient sorghum lines grown under dry land and irrigated conditions at Colby, KS in 2007

		Type III Mean Squares
Source of variation	**df**	**One Meter Biomass**
Rep	3	125870.8
Irr	1	71593.9
Error a	3	480717.6
Line	7	247173.7
Irr*Line	7	282080.0
Error B	29	176956.4
Low TE vs High TE	1	1364300.3

*, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Table 2.23 Mean value of final one meter row harvest data studied in high and low transpiration efficient sorghum lines evaluated under field lysimeter condition studied under irrigated condition at Colby, KS in 2007

	Least Square Means	
	One Meter Biomass (g)	
	Irr	Dry
Low TE		
PI 257309	1640.8 a	1876.9a
PI 295121	1620.2a	832.1c
PI 586381	1237.1a	1700.6ab
PI 267532	1231.8a	1328.2abc
Mean	1432.5	1434.5
High TE		
PI 567933	1099.0 a	1350.0abc
PI 391652	878.4a	1211.7abc
PI 533946	949.24a	1051.2bc
PI 584085	1071.1a	1076.0bc
Mean	999.4	1172.2
Critical Value	891	689
†† Low TE Vs High TE	ns	ns

†Least squares means followed by the same letter do not differ at the 0.05 level of probability, DMRT

††Linear contrast testing against null effects of low TE and high TE lines; *, **, *** indicate significance at P<0.05, p<0.01 and p<0.001, respectively

Discussion

Greenhouse Study (Manhattan)

High TE plants emerged better than low TE plants, which indicated that high TE plants have a physiological mechanism that allows faster growth and penetration through the soil than low TE plants. More of the low TE plants than high TE plants died in the soil before they could emerge. More research needs to be done to determine the physiological mechanisms that control vigor in high and low TE seeds.

The plant water relations (relative water content, stomatal resistance, pressure potential) of low TE plants were similar to those of high TE plants. Great variability in the measurements prevented significant differences. The number of seeds available for experimentation precluded more replications, which might have reduced the variability. Also, the relatively poor emergence of all experimental plants limited the number of measurements. There was a tendency for the low TE plants to have a lower stomatal resistance under dry conditions than the high TE plants under dry conditions. Further experiments are needed to verify this observation.

At the end of the experiment, low TE plants grew as well as high TE plants. This indicated that once the plants emerged, low and high TE plants grew the same. However, the fresh weight of the high TE plants that were well watered was higher than that of the high TE plants that were drought-stressed. The fresh weights of the low TE plants under the two watering regimes were similar.

In conclusion, the data indicated that to screen plants for low or high TE efficiency, emergence should be documented. Plants that emerge poorly may have a low TE efficiency. The water relations of low and high TE plants were similar in this experiment, and the data suggested that measurements of relative water content, stomatal resistance, or pressure potential cannot be used to distinguish high and low TE plants. More research needs to be done on the seed physiology of low and high TE plants to determine why their seeds differ in vigor.

Field lysimeter and field study (Colby and Hays)

The difference seen at in the growth measurement taken at the harvest at Hays, shows that these genotypes are significantly different in their growth and development. The important is that, these genotypes were different in their reproductive stage. Some lines keep adding leaves delaying the reproductive stage and some just opposite, comparatively quick to reproductive stage. This type of variation indicates they are not adaptive to the climate and there is difference in photoperiod sensitive.

The results (Table 2.13, Table 2.15 and Table 2.17) from the experiment show difference in A and g_s among lines. The results indicate that there is strong relationship between assimilation (A), stomatal conductance (g_s), and transpiration (T). The interesting thing is two lines showed difference in A and g_s in all three gas exchange measurements.

Carbon dioxide and water vapor share a common pathway between the leaf mesophyll and the atmosphere, resulting in a relatively high correlation between assimilation and conductance. Tanner and Sinclair (1983) have stated that due to this high correlation there is little opportunity to increase A without increasing g_s. However, assimilation and conductance respond independently to environmental factors such as vpd, solar radiation, and temperature. Since CO_2 supply has little effect on g_s and A, and also g_s and A are independent of environment factor, any difference in transpiration efficiency or water use efficiency may be due to the difference in assimilation and stomatal conductance. So from the previous studies (Kidambi et al. 1990; Blum and Sullivan 1972; Krieg and Hutmacher 1982) carried with leaf gas exchange measurements suggest that plant varying in stomatal conductance and carbon assimilation show difference in transpiration efficiency and water use efficiency.

The special features of stomata and their role in regulation of water loss have been recognized for many years. Stomata must be the primary control of transpiration (Bange 1953). Our results support this statement. The stomatal role in controlling transpiration may be quantified as the relative change of transpiration rate corresponding to a relative change in stomatal conductance. Under our standard measurement conditions [C_a, temperature (cuvette), relative humidity (outside the leaf) vpd and PPFD were kept constant], the primary difference in transpiration in the leaf is due to differences in leaf stomatal conductance. With the same driving gradient and with the same temperature, the leaf with greater stomatal conductance would have

greater transpiration. These experimental results show corresponding differences in g_s and T among lines.

Assimilation is linked to light utilization. The flow of electrons from photosystem II provides energy to drive assimilation (Maxwell and Johnson 2000). So increase electron flow is expected to result in increase in assimilation rate which is expected to result in an increase in g_s. Once the efficiency of photosystem II increases, the concentration of CO_2 in the leaf decreases causing the stomata to open, which results in high stomatal conductance.

Another important factor is the internal carbon dioxide in the leaf, which also plays a key role in TE. The carbon dioxide assimilation system in C_4 photosynthesis allows increased photosynthetic rates at small internal carbon dioxide concentrations, permitting greater photosynthetic rates relative to stomatal conductance (g_s) than in C_3 plants (Bunce 2005). Consider two leaves differing in C_i but identical in ambient CO_2 (C_a) and stomatal conductance. The CO_2 driving gradient will be greater for the leaf with small C_i. In consequence, the assimilation will be greater in the leaf with small C_i under these conditions. Since assimilation is greater in the leaf with small C_i and the transpiration similar for both leave, (assuming identical leaf temperature), transpiration efficiency would be greater in the leaf with low C_i. Similarly the difference in dark internal carbon dioxide explains that some of the lines use the stored C_i during night reducing the physiological mechanism for production of biomass.

In conclusion, there was difference in the leaf gas exchange measurement with two lines (PI295121 and PI533946) showing consistent difference on certain physiological mechanism. No consistent difference in biomass and water use were be detected. More research needs to be concentrated looking for relationship between growth and development with biomass and water use, because of the photoperiod sensitivity expressed by these lines. And finally, developing reliable selection indices for TE will require a greater understanding of whole-plant physiological processes to utilize the differences in TE observed at the leaf level.

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