QUANTITATIVE TRAIT LOCUS (QTL) MAPPING OF TRANSPIRATION EFFICIENCY RELATED TO PRE-FLOWER DROUGHT TOLERANCE IN SORGHUM [*Sorghum bicolor* (L.) MOENCH]

A Dissertation

by

MOHANKUMAR HERAGANAHALLY KAPANIGOWDA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2011

Major Subject: Plant Breeding

Quantitative Trait Locus (QTL) Mapping of Transpiration Efficiency Related to Pre-flower Drought Tolerance in Sorghum [*Sorghum bicolor* (L.) Moench]

QUANTITATIVE TRAIT LOCUS (QTL) MAPPING OF TRANSPIRATION EFFICIENCY RELATED TO PRE-FLOWER DROUGHT TOLERANCE IN SORGHUM [*Sorghum bicolor* (L.) MOENCH]

A Dissertation

by

MOHANKUMAR HERAGANAHALLY KAPANIGOWDA

Submitted to the Office of the Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Co-Chairs of Committee,	William A. Payne
	William L. Rooney
Committee Members,	John E. Mullet
	Seth C. Murray
	Maria M. Balota
Head of Department,	David Baltensperger

May 2011

Major Subject: Plant Breeding

ABSTRACT

Quantitative Trait Locus (QTL) Mapping of Transpiration Efficiency Related to Pre-flower Drought Tolerance in Sorghum [*Sorghum bicolor* (L.) Moench]. (May 2011)

Mohankumar Heraganahally Kapanigowda, B.S., Univeristy of Agricultural Sciences, Bangalore, India;

M.S., University of Agricultural Sciences, Bangalore, India

Co-Chairs of Advisory Committee: Dr. William A. Payne
Dr. William L. Rooney

There is an increasing need to improve crop water-use efficiency (WUE) (ratio of whole-plant biomass to cumulative transpiration) due to decreased water availability and increased food and energy demands throughout the world. The objective of the study was to estimate the genetic variation and genetic basis for transpiration efficiency A:E (CO_2 assimilation rate (A) divided by transpiration rate (E)) trait and its relationship to WUE related to pre-flower drought tolerance in recombinant inbred lines (RILs) of sorghum and associated QTLs. A greenhouse study was conducted at Bushland, TX, 2008, using 71 RILs derived from cross of Tx430 x Tx7078. A randomized complete block experimental design was used, with both genotype and water regime (40 and 80% water regime) as experimental factors, and four replications. Genotype had a significant effect on A, E and A:E under both the environments. Among the RILs, entry means for A:E ranged from 1.58 to 3.07 mmol CO_2 mol^{-1} H_2O and 1.18 to 4.36 mmol CO_2 mol^{-1} H_2O under 80% and 40% water regime, respectively. Heritability estimates based on

individual environments for A:E , A and E were 0.77, 0.45 and 0.37 under 80% water regime and 0.90, 0.33 and 0.71 under 40% water regime, respectively. A genetic map was constructed by digital genotyping method using Illumina GAII sequencer with 261 informative indel/ single-nucleotide polymorphism (SNP's) markers distributed over 10 linkage groups. Three significant QTLs associated with transpiration efficiency were identified; two on SBI-09 and one on SBI-10 with one logarithmic of odds (LOD) interval length ranging from 5.3 to 5.7 cM and accounting for 17% - 21% of the phenotypic variation. In field and greenhouse evaluation of agronomic of traits at College Station and Halfway, TX, 91 QTL that control variation in six major agronomic traits such as plant height, flowering, biomass, leaf area, leaf greenness and stomatal density were identified. Co-localization of transpiration efficiency QTLs with agronomic traits such as leaf area, biomass, leaf width and stomatal density indicated that these agronomically important QTLs can be used for further improving the sorghum performance through marker assisted selection (MAS) under pre-flowering drought stress conditions.

I dedicate this dissertation to my beloved wife, Divya Gowda.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my committee co-chairs, Dr. Bill Payne and Dr. Bill Rooney, who brought me to Texas A&M University and allowed me to obtain my Ph.D. under their guidance. I heartily appreciate their constant support, encouragement and invaluable guidance throughout my Ph.D. process and also in obtaining plant breeding experience. I am also heartily thankful to my committee member, Dr. John Mullet, for his support, valuable input to my research and providing me the facilities to carry out the genotyping of the population. I would also like to thank my other committee members, Dr. Seth Murray and Dr. Maria Balota, for their support and valuable input to my dissertation research. My appreciation and thanks also go to Dr. Daryl Morishige and Dr. Patricia Klein for their help with running the sequencer and analyzing the preliminary sequence data. I would like to thank Susan Hall, Brock Weirs, and Sara for their assistance during the genotyping and QTL analysis. I also would like to thank all the members and my colleagues of the Sorghum Breeding group for their friendship and constant support throughout the study. Special thanks to Dr. Bob Stewart, WTAMU, for his affection, support and guidance which I cannot forget in my life.

I would like to thank my father, the late Kapanigowda, and mother, Parvathamma for their love and affection, trust and motivation towards my education. I am also thankful to all my family members for their moral support and best wishes extended to me and who missed my presence at home. My special thanks to my sweetheart, Divya Gowda, for her love, support and encouragement in finishing my dissertation.

The financial support from Great Plains Sorghum Initiative Project and McKnight foundation, Norman Borlaug Institute for International Agriculture are thankfully acknowledged.

Lastly, I offer my regards to all of those who supported me in any respect during my study.

TABLE OF CONTENTS

Page

Page

LIST OF FIGURES

FIGURE Page

LIST OF TABLES

TABLE Page

CHAPTER I

GENERAL INTRODUCTION

Globally, agriculture consumes over 70 percent of fresh water resources every year (Bacon, 2004). United Nations projected that by 2050, world population will increase to 9.1 billion from 6.8 billion today (United Nations, 2011). With the rapid increase in population and scarcity of the fresh water resource, water shortage (or drought) has become the key factor that constrains crop production worldwide. Due to these limitations there are urgent needs to increase the yield and water use efficiency under water limiting conditions (Hamdy et al., 2003).

Drought tolerance is an extremely complex trait that is dependent on many factors, which include but are not limited to the weather, the timing and the severity of moisture stress. Progress in developing drought tolerant germplasm through plant breeding is limited by an incomplete understanding of the genetic and physiological mechanisms that condition its expression. Even though considerable work has been done on plant response to moisture stress (Tuinstra et al., 1996; Xu et al., 2000; Kebede et al., 2001; Haussmann et al., 2002; Borrell et al., 2004), there has been little emphasis on the use of specific physiological traits to enhance the drought stress tolerance. Specific physiological trait like transpiration efficiency, which is defined as change in CO_2

This dissertation follows the style of Crop Science.

assimilation rate (A) per unit change in transpiration rate (E), is one potential way to increase water productivity under drought stress condition.

Worldwide in 2009, sorghum (*Sorghum bicolor* (L.) Moench) was harvested in an area of 43.7 m ha with average yield of 1.41 metric tons per hectare, which makes it the fifth most important cereal crop after wheat, maize, rice and barley (FAO, 2009). Sorghum is a staple food crop for millions of people in arid and semiarid regions, primarily in Africa and parts of Asia. In terms of utilization, worldwide, almost half of the grain sorghum produced is used as animal feed. More recently sorghum has also been proposed as a dedicated cellulosic bioenergy feedstock (Rooney et al., 2007).

Sorghum is known for its extensive phenotypic and genotypic variation in response to drought (Blum, 1979; Doggett, 1988). The superior drought tolerance in the crop is likely due to its evolution in Sub-Saharan Africa, a region charecterized by low and erratic rainfall patterns. Thus, it is believed to be one of the most drought tolerant crops and an good model for studying the genetic and physiological mechanisms of drought tolerance.

Potential drought tolerance mechanisms in plants include drought escape, drought (dehydration) avoidance and dessication (drought) tolerance (Blum, 1988). Drought response in sorghum has been classified into two distinct stages, pre-flowering and post-flowering (Rosenow, 1987). Pre-flowering drought stress leads to leaf rolling and erectness, delayed flowering and reduced height. On the other hand, post-flowering drought stress causes premature leaf senescence leading to stalk lodging, stalk rot disease and significant yield loss (Rosenow and Clark, 1995). Understanding the

genetics and physiology of pre-flower drought tolerance and its related agronomic traits has the potential to develop drought tolerant sorghum.

Several studies have reported that sorghum possesses genetic variation for many traits, including transpiration efficiency, related to drought tolerance (Krieg and Hutmacher, 1986; Kidambi et al., 1990a, Peng et al., 1991; Peng and Krieg, 1992; Krieg et al., 1992, Balota et al., 2008). However, selection for drought tolerance while maintaining high productivity has been a great challenge (Rosenow et al., 1983).

With the advancement of molecular genotyping and QTL mapping techniques, it is now possible to dissect genetic factors that contribute to a complex trait like drought tolerance (Paterson et al., 1988). Several studies (Tunistra et al., 1997; Crasta et al., 1999; Subudhi et al., 2000; Xu et al., 2000; Harris et al., 2007) have characterized and mapped post-flowering drought tolerance, including the stay green trait, using various sorghum populations. Stay-green plants retain chlorophyll in their leaves and maintain the ability to carry out photosynthesis longer than 'senescent' genotypes under terminal drought conditions. Harris et al. (2007) mapped the stay green trait to four major QTLs (*Stg1-Stg4)* that individually reduces the post-flowering drought induced leaf senescence using a population derived from cross of BTx642 x RTx7000.

In the US Great Plains and elsewhere, water stress occurs most frequently during pre-flowering stage due to low precipitation, low humidity and high evaporative demand (Bandaru et al., 2006). Even though pre-flowering drought-stress commonly occurs in sorghum production environments (Rosenow et al., 1996), very few genetic analyses have been completed for pre-flowering drought-stress. Tuinstra et al.(1996) identified

six regions in the sorghum genome associated with pre-flowering drought tolerance using recombinant inbred lines derived from cross between Tx7078 x B35, which are resistant and susceptible, respectively to pre-flower drought. However, additional reports on the control of pre-flowering drought stress are limited (Rosenow et al., 1996).

Given the dearth of information on pre-flowering drought-stress, this study was undertaken with the following objectives: (1) To study the genetic variation for transpiration efficiency (A:E) related to pre-flower drought tolerance in a sorghum (*Sorghum bicolor* L. Moench) RIL population under controlled conditions at leaf level and whole plant level; (2) To identify the QTL that condition traits associated with pre-flowering drought stress using a RIL population derived from the cross Tx430 x Tx7078; and (3) To understand the genetic and physiological determinants of water use by relating agronomic traits to transpiration efficiency.

CHAPTER II

GENETIC VARIABILITY FOR GAS EXCHANGE RATES AND TRANSPIRATION RATIO RELATED TO PRE-FLOWER DROUGHT TOLERANCE IN SORGHUM

2.1 Introduction

Globally in 2000, agriculture accounted for 67% of the world's total fresh water withdrawal and 86% of its consumption. By 2025 agriculture is expected to increase its water requirement by 1.3 times this level (UNESCO, 1998). Increased demand of rapidly declining fresh water supplies, coupled with the need to meet increased demand for food associated with a projected world population of 9.1 billion by 2050 (United Nations, 2011), poses a great challenge to agriculture. There is an increasing need to improve crop water-use efficiency (WUE) (i.e., the ratio of whole-plant biomass to cumulative transpiration) due to decreasing water availability and increasing food and energy demands throughout the world (Balota et al., 2008). In most developing countries, on-farm water use efficiency is very poor, approaching only 45 percent of theoretically optimal values (FAO, 2002). Recent improvements in irrigation technology and other conservation agriculture practices that reduce water loss through soil surface water evaporation and runoff have played significant roles in increasing water productivity (Howell, 2001). Increasing the transpiration efficiency defined as the biomass produced per unit water transpired, is another important option to increase the water productivity (Condon et al., 2004).

Sorghum [*Sorghum bicolor* (L.) Moench] is the world's fifth most important grain crop based on production, after maize, wheat, rice, and barley (FAO, 2009) and provides staple food for millions of people in semi-arid tropics of Africa and Asia. In 2009, sorghum was produced on approximately 40.23 million hectares with an average yield of 1.49 metric tons per hectare worldwide (USDA, 2010). It is also one of the most drought tolerant (Blum, 2004) and water-efficient cereals, and therefore well adapted to semi-arid tropical and subtropical environments (Kidambi et al., 1990a; Rooney, 2004).

Sorghum is also an important source of "green" energy. It can be used as lignocellulosic as well as starch based source for biofuel production. Currently, it is a distant second to maize as a starch source for ethanol production in US after maize. As a C_4 crop, sorghum possesses high transpiration efficiency (Xin et al., 2009). Furthermore, it has a highly diverse source of useful alleles (Rosenow and Dahlberg, 2000), which offers the potential to breed for even more drought tolerant and water efficient genotypes.

Several field and greenhouse studies have found significant genetic variation for transpiration ratio [CO_2 assimilation rate (A) /transpiration rate (E), A:E], when measured using the LICOR 6400 Infrared gas analyzer (IRGA)] in grain sorghum and under water limited conditions (Krieg and Hutmacher, 1986; Kidambi et al., 1990a, Peng et al., 1991; Peng and Krieg, 1992; Krieg et al., 1992; Balota et al., 2008). Significant variation in the ratio of A to stomatal conductance (g) has been observed (Kidambi et al. (1990a). In their study, g was relatively conservative, suggesting that it may be possible to select for increased A without a concurrent increase in g in sorghum. Genotypic

variation in A was associated with increased leaf area and shoot biomass production; this occurred without significant increase in water use or leaf transpiration (Peng and Krieg (1992). Further, they concluded that measurements of A and leaf area could be used as selection criteria for higher WUE in grain sorghum under field conditions. Peng et al. (1991) reported a strong correlation between A and total biomass production in 22 sorghum genotypes. They also suggested that single leaf measurement of A could be used to select for higher productivity among sorghum genotypes. Balota et al. (2008) examined four sorghum parental inbred lines and 12 of their hybrids for transpiration ratio under water limited and well watered conditions. They found that average A:E over both water conditions was 3.10 mmol CO_2 mol^{-1} H_20 for Tx430 and 2.91 for Tx7078. These two genotypes also had the highest A. They concluded that there is genetic variation for pre-flower A, E, and A:E rating as well as WUE in sorghum genotypes. Further, they revealed from their data that greater transpiration ratio may be one component of pre-flowering drought tolerance during 8- 12 leaf stage of plant development (GS2 growth phase), which plays an important role determining the yield in the U.S. Great Plains (Eastin et al., 1983). However, they suggested further investigation of the relationships among and genetic control of A, A:E, and WUE to elucidate genetic and environmental control over these traits.

Significant genetic variation for WUE in sorghum has been reported in numerous studies conducted across various environments (Bhargava et al., 2004; Hammer et al., 1997; Henderson et al., 1998; Mortlock and Hammer, 1999; Donatelli et al., 1992). Hammer et al., (1997) concluded that genetic variation observed among 49 diverse lines

of sorghum and one weedy species (*Sorghum halepense*) warranted further study on transpiration efficiency. Their study was conducted under non-limiting water and nutrient conditions using a semi-automatic pot watering system; however, they suggested that screening for genetic variation under water limited conditions could provide useful insights to transpiration efficiency. Mortlock and Hammer (1999) found that among 17 sorghum genotypes, the transpiration efficiency of the best performing genotype was 50% greater than the least efficient genotype. They also observed a 9% increase in the transpiration efficiency of plants under water limited conditions compared to those under fully watered conditions. Donatelli et al. (1992) found significant effects of genotype and water supply among six sorghum genotypes. Relative to fully watered plants, transpiration efficiency of water limited plants increased by 28%.

Even though pre-flowering drought-stress commonly occurs in sorghum production environments (Rosenow et al., 1996), very few genetic analyses have been completed for pre-flowering drought-stress. Thus, there is little information on the physiology and genetics of pre-flowering drought tolerance. This study was undertaken using a recombinant inbred lines (RILs) derived from the cross of Tx430 x Tx7078 with the following objectives: (1) To study the genetic variation for transpiration efficiency (A:E) related to pre-flower drought tolerance in sorghum (*Sorghum bicolor* L. Moench) recombinant inbred lines population under controlled conditions at leaf level and whole plant level; and (2) To estimate the heritability of transpiration efficiency related traits.

2.2 Material and Methods

A total of 70 F_6 generation recombinant inbred lines (RILs) and two parents (Tx430 and Tx7078) were developed in the Texas AgriLife Breeding Program directed by Dr. William L. Rooney. The parents were selected based on contrasting values of transpiration ratio (A:E). Balota et al. (2008) observed that Tx430 had high A and high A:E, while Tx7078 had high A and low A:E. The RILs and their parents were grown in a greenhouse at the Texas AgriLife Research and Extension Station in Bushland, TX, (35°11' N lat; 102°06' W long; 1170 m elevation) to measure gas exchange and WUE. Air temperature and relative humidity were continuously monitored at plant canopy level with a temperature / relative humidity probe and data logger (model HMP45C, Campbell Scientific Inc, Logan, UT). The experimental design was a randomized complete block with four replications, with genotype and water regime (40 and 80 percent water regime) as experimental factors. Each replication was planted with an interval of one week to facilitate the consistency in measurements of gas exchange and WUE. The first replication was planted on June 20th, 2008 in pots using four seeds per pot. After emergence, pots were thinned to one plant per pot which served as an experimental unit.

Each pot contained 2.5 kg finely screened Pullman clay loam soil (fine, mixed, superactive, thermic Torrertic Paleustolls). Nutrient content and chemical properties of the Pullman soil are shown in Table 2.1. The two water treatments i.e. 80 % and 40% water regime were based on soil water retention curves and correspond to soil water content of 0.18 kg water kg^{-1} soil and 0.09 kg water kg^{-1} soil, respectively.

Plants were fertilized with 110 mg N, 66 mg P_2O_5, 88 mg K_2O, 6 mg Mg, 12 mg S, 0.007 mg B and Mo, 0.18 mg Cu and Zn, 0.22 mg Mn, and 1.65 mg Fe kg^{-1} soil with Osmocote Plus commercial fertilizer (Scotts-Sierra Co., Marysville, OH) at 10 days after planting.

Each pot was lined with a plastic bag, which was wrapped around the stems to minimize water evaporation from the soil surface. For the first 15 days after planting (DAP) all the pots were maintained under well watered conditions. Water treatments were imposed at 16 d after planting and maintained by daily weighing and watering, using an electronic balance with increments of 5 g. Daily transpiration per plant was calculated as the difference between initial and final pot mass. Daily transpiration was summed to give cumulative transpiration per plant. Total biomass per plant (roots and shoots) was obtained at 35 days after planting. Shoots were cut at soil level and roots were gently washed on a 1-mm sieve. Roots and shoots were then oven dried at 60 °C and weighed. WUE was calculated as total biomass divided by cumulative transpiration from planting to harvest.

Table 2.1: Nutrient contents and chemical properties of soil used as a potting mixture for the experiment at Bushland, TX.

Soil pH	Soluble Salts	Organic Matter	Nitrate- N	P	K	S	Ca	Mg	Na	Zn	Fe	Mn	Cu
	mmho/cm	g kg^{-1}	----------mg ka^{-1}----------										
7.9	0.59	10.0	37	33	398	18	4308	370	45	0.8	7	9	0.95

2.2.1 Gas Exchange Measurements

Gas-exchange measurements were taken with the LI-6400 Infrared Gas Analyzer (IRGA) portable photosynthesis system (LI-COR, Lincoln, NE) on 70 inbred lines and two parents. During measurements, photosynthetic photon flux density (PPFD) recorded at plant canopy level was 1700 ± 200 μ mol m^{-2} s^{-1}. A high flow rate of 500 μ mol s^{-1} was used to keep humidity inside the chamber at less than 2% variation. A 6400-01 CO_2 mixer (LI-COR, Lincoln, NE) was used to inject and maintain a constant concentration of 400 μ mol CO_2 mol^{-1} air during measurements. Leaves were allowed to equilibrate for 60 s before each reading of A and E. Transpiration efficiency (A:E) was calculated in two ways: as a change in CO_2 assimilation rate (A) per unit change in transpiration rate (E) divided by vapor pressure deficit (VPD) at the leaf surface (VPD was measured inside the leaf chamber of the LICOR-6400 IRGA during the gas exchange measurement) and as the regression slope of A vs. E. The uppermost fully developed leaves were measured between 1200 and 1400 h on four successive days starting at 30 days after planting (Figure 2.1).

Figure 2.1. Measurement of the transpiration efficiency traits on the topmost fully opened sorghum leaf using the LI-COR 6400 Infrared Gas Analyzer.

2.2.2 Statistical Analysis

Genotype effect on A, E, and A:E was analyzed with ANOVA from the GLM procedure of SYSTAT 10.2 (2002, SYSTAT Software Inc., Richmond, CA) using genotype and replication as independent variables and each pot as an experimental unit. Simple linear regression equations were fitted to A vs. E divided by vapor pressure deficit (EVPD) as well as total biomass and cumulative transpiration per plant to evaluate the effect of RILs and parents on the slopes of A vs EVPD and biomass vs. cumulative transpiration, similar to the procedure followed by Balota et al. (2008). Frequency distribution of A:E, A and E at 80% and 40% water regime in 70 recombinant inbred lines and two parents were obtained based on their mean values. Frequency distribution graph for A:EVPD

slope values was obtained by regressing CO_2 assimilation rate (A) vs. transpiration rate (EVPD) in 70 RILs to estimate the genetic variation among the lines. Pearson correlation coefficients were obtained for A, E and A:E as well as for whole plant WUE to evaluate the relationship of recombinant inbred lines on leaf level and whole plant level water use efficiency.

Variance components for genotype (G) with regards to A, E and A:E were obtained from ANOVA by analyzing the individual environment (water regime) using SYSTAT 10.2 software and the trait σ_G^2 was estimated as :

$$\sigma_G^2 = (MS_G - MS_e) / r$$

where, σ_G^2 is variance due to genotype, MS_G is the mean square of genotype, MS_e is the mean square of error and 'r' is the number of replications. For predicting effects genotypes were treated as random effects. These variance components were used to calculate broad-sense heritability:

$$H^2 = \sigma_G^2 / (\sigma_G^2 + \frac{\sigma_e^2}{r})$$

where 'σ_e^2' is the variance due to error and 'r' is the number of replications.

Exact 90% (1-α = 0.90) confidence limit were determined for heritability similar to the method followed by Knapp (1985) using the formula:

$$1 - [(MS_G / MS_e) F_{\alpha/2 : df_2, df_1}]^{-1} = 1 - \alpha$$

Analysis over the two environments (water regime) were not combined, as genotype had no significant effect on A:E using random effect model. Moreover, QTLs were also identified separately under each individual environment.

2.3 Results and Discussion

2.3.1 Weather

Gas-exchange measurements were carried out under near optimum air temperature and atmospheric humidity for sorghum growth and development. In the greenhouse, average air temperature during the crop growth period was 30^0C and 21^0C during day and night, respectively, which is an ideal temperature to achieve maximum photosynthesis (Bennett et al, 1990). Average relative humidity was maintained at 50 percent, and the average VPD was 2.3 kPa (Figure 2.2).

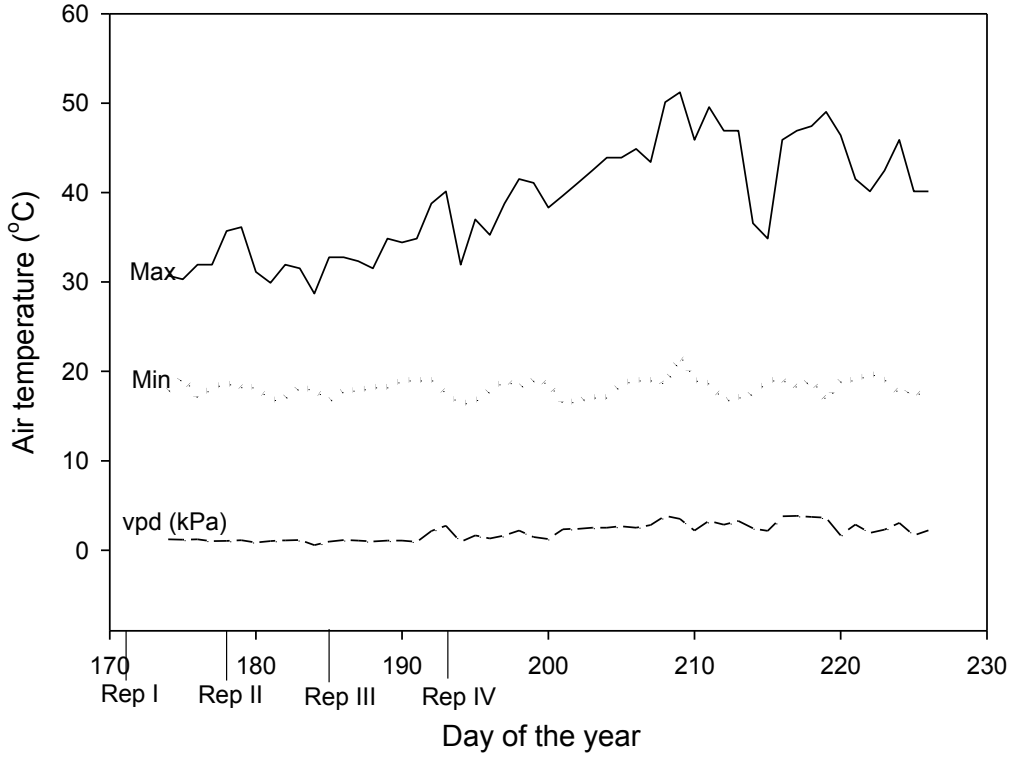

Figure 2.2. Summary of the weather data from greenhouse, Bushland, TX from 19th June to 18th August (vpd: vapor pressure deficit). Rep I, Rep II, Rep III and Rep IV represent the date of planting for each replication.

2.3.2 Phenotypic and Genetic Variation

Genotype had a highly significant effect on A ($P < 0.0001$ and $P<0.0001$), E ($P < 0.002$ and $P<0.007$) and A:E ($P < 0.0001$ and $P<0.0001$) under 80% and 40% water regimes, respectively (Table 2.2). Among the RILs, entry means for A ranged from 27.46 to 42.3 µmol CO_2 m^{-2} s^{-1}, E from 6.87 to 10.08 mmol H_2O m^{-2} s^{-1} and and A:E from 1.58 to 3.07 mmol CO_2 mol^{-1} H_2O and under 80% water regime (Table 2.3). Under 40% water regime, entry means for A ranged from15.6 to 48.7 µmol CO_2 m^{-2} s^{-1}, E from 3.71 to

Table 2.2. Mean squares and probability levels (*p*) by environment (water regime) from ANOVAs for gas exchange traits related to pre-flower drought tolerance in sorghum RIL population derived from Tx430 x Tx7078, Bushland, Texas during 2008.

Source	Df	A ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)		E ($mmol\ H_2O\ m^{-2}\ s^{-1}$)		A:E ($mmol\ CO_2\ mol^{-1}\ H_2O$)	
		Mean square	*p*	Mean square	*p*	Mean square	*p*
80% Water regime							
Genotype	72	70.86	<0.0001	5.40	0.0022	0.88	<0.0001
Replication	3	823.26	<0.0001	383.87	<0.0001	132.85	<0.0001
Error	681	38.75		3.40		0.19	
40% Water regime							
Genotype	72	114.06	<0.0001	4.47	0.0073	1.02	<0.0001
Replication	3	4146.86	<0.0001	43.57	0.0001	116.96	<0.0001
Error	601	32.28		2.99		0.09	

Table 2.3. Mean A, E and A:E for Tx430 x Tx7078 parental and recombinant inbred lines (RILs) at greenhouse, Bushland, Texas during 2008.

	Tx430 (SD)†		Tx7078		RILs mean		RILs range	
Trait	40% FC	80%FC	40% FC	80%FC	40% FC	80%FC	40% FC	80%FC
CO2 Assimilation rate (A) (μmol CO_2 m^{-2} s^{-1})	30.84 (2.17) †	38.37 (2.86)	30.56 (7.37)	35.84 (6.84)	30.12 (4.14)	37.21 (2.68)	15.6-48.7	27.46-42.3
Transpiration (E) mmol H_2O m^{-2} s^{-1})	6.71 (0.94)	8.42 (1.54)	7.40 (1.14)	8.82 (1.87)	6.65 (0.73)	8.37 (0.72)	3.71-8.99	6.87-10.08
Transpiration ratio(EVPD) (mmol CO_2 mol^{-1} H_2O)	1.81 (0.27)	2.34 (0.74)	1.60 (0.25)	2.03 (0.55)	1.93 (0.44)	2.29 (0.31)	1.18-4.36	1.58-3.07

†Standard deviation in parenthesis.

8.99 mmol H_2O m^{-2} s^{-1} and A:E from 1.18 to 4.36 mmol CO_2 mol^{-1} H_2O. Genetic variation in A and E among sorghum lines and their hybrids have been reported previously by several authors (Kidambi et al., 1990b: Peng and Krieg, 1992; Balota et al., 2008). Kidambi et al. (1990a), reported substantial genetic variation in the A: g (stomatal conductance) relationship that caused by significant genetic variation in A. However, g was relatively more conservative to increasing water stress. They proposed that selection for high A might directly contribute to greater WUE and higher drought tolerance. However, Balota et al. (2008), suggested that concomitant selection for high A:E and A may be necessary when high biomass and WUE are both desired. In our study, we found genotypes had highly significant effect on A, E and A:E, which provides further evidence that when we desired to have potentially greater drought tolerance, it might be necessary to select for both higher A:E and A.

As expected, 80% water regime had higher overall mean values for A, E, and A:E among the RILs compared to 40% water regime (Table 2.3). Among the parents, under 80% water regime, Tx430 had a higher mean A (38.37 µmol CO_2 m^{-2} s^{-1}) and A:E (2.34 mmol CO_2 mol^{-1} H_2O) compared to Tx7078 with mean A (35.84 µmol CO_2 m^{-2} s^{-1}) and A:E (2.03 mmol CO_2 mol^{-1} H_2O). In 40% water regime mean A and A:E were 30.84 µmol CO_2 m^{-2} s^{-1} and 1.81 mmol CO_2 mol^{-1} H_2O, respectively, for Tx430 and 30.56 µmol CO_2 m^{-2} s^{-1}) and 1.60 mmol CO_2 mol^{-1} H_2O, respectively, for Tx7078. These results are consistent with other field observations (Kidambi et al.,1990b; Peng et al., 1991; Peng and Krieg 1992; and Balota et al., 2008). However, Tx7078 had slightly higher transpiration rate E (8.82 and 7.40 mmol H_2O m^{-2} s^{-1}) compared to Tx430 (8.42

and 6.71 mmol H_2O m^{-2} s^{-1}) in both 80% and 40% water regimes, similar to the results reported by Balota et al. (2008).

The recombinant inbred lines for mean values of A:E, A and E were continuously distributed, as expected for a quantitative trait both under 80% and 40% water regimes except in A:E under 40% water regime (Figure 2.3). The normal distribution of these traits indicated polygenic segregation. However, skewed distribution of A:E under 40% water regime suggested the involvement of a single gene with large effects controlling this physiological trait.

The mean regression slope for A vs. EVPD was 2.76 mmol mol^{-1} for RILs under 80% water regime and 4.84 mmol mol^{-1} under 40% water regime (Figure 2.4). Among the parental lines, Tx430 (5.53 mmol CO_2 mol^{-1} H_2O) had higher slope of A vs. EVPD compared to Tx7078 (2.53 mmol CO_2 mol^{-1} H_2O) under 80% water regime. However, under 40% water regime, Tx7078 (8.70 mmol CO_2 mol^{-1} H_2O) had higher slope of A vs. EVPD compared to Tx430 (6.23 mmol CO_2 mol^{-1} H_2O) (Figure 2.4). Our results are therefore consistent with those of Kidambi et al. (1990b) and Balota et al.(2008), who found that Tx430 had the greatest A and A:E under various water regimes. Balota et al. (2008) observed that Tx430 hybrids used ~30% less water to fix the same amount of CO_2 compared to similar Tx7078 hybrids.

A frequency distribution graph for slope values obtained by regressing A on EVPD showed a greater genetic variability among the inbred lines than among parents for transpiration efficiency (A:E) (Figure 2.5), indicating greater transgressive segregation among progeny. This might be due to the greater combination of favorable alleles for the trait from both the parents and also might be due to epistatic interaction between the alleles.

2.3.3 Trait Heritability

Broad-sense heritability estimates for A:E, A and E were 0.77, 0.45 and 0.37 for RILs under 80% water regime and 0.90, 0.33 and 0.71 under 40% water regime (Table 2.4). These heritabilities provide evidence that there is likely selectable genetic variability among genotypes for gas exchange rates at pre-flowering in sorghum that suggest WUE could be improved through selection and breeding. These heritability estimates are almost equivalent to narrowsense heritability, since genetic variance in F_6 population is almost equal to additive genetic variance as dominance variance is negligible.

Table 2.4. Trait heritability based on individual environment (water regime) in sorghum RIL population with four replications, Bushland, Texas during 2008.

Trait	Heritability	
	40% FC	80% FC
Transpiration efficiency (A:E)/vpd (mmol CO_2 mol^{-1} H_2O)	0.90 (0.88-0.93)	0.77 (0.69-0.83)‡
CO_2 assimilation rate (A) (μmol CO_2 m^{-2} s^{-1})	0.33 (0.09-0.49)	0.45 (0.14-0.52)
Transpiration rate (EVPD) (mmol H_2O m^{-2} s^{-1})	0.71 (0.61-0.78)	0.37 (0.25-0.58)

‡ Exact 90% (1- α=0.90) confidence limit.

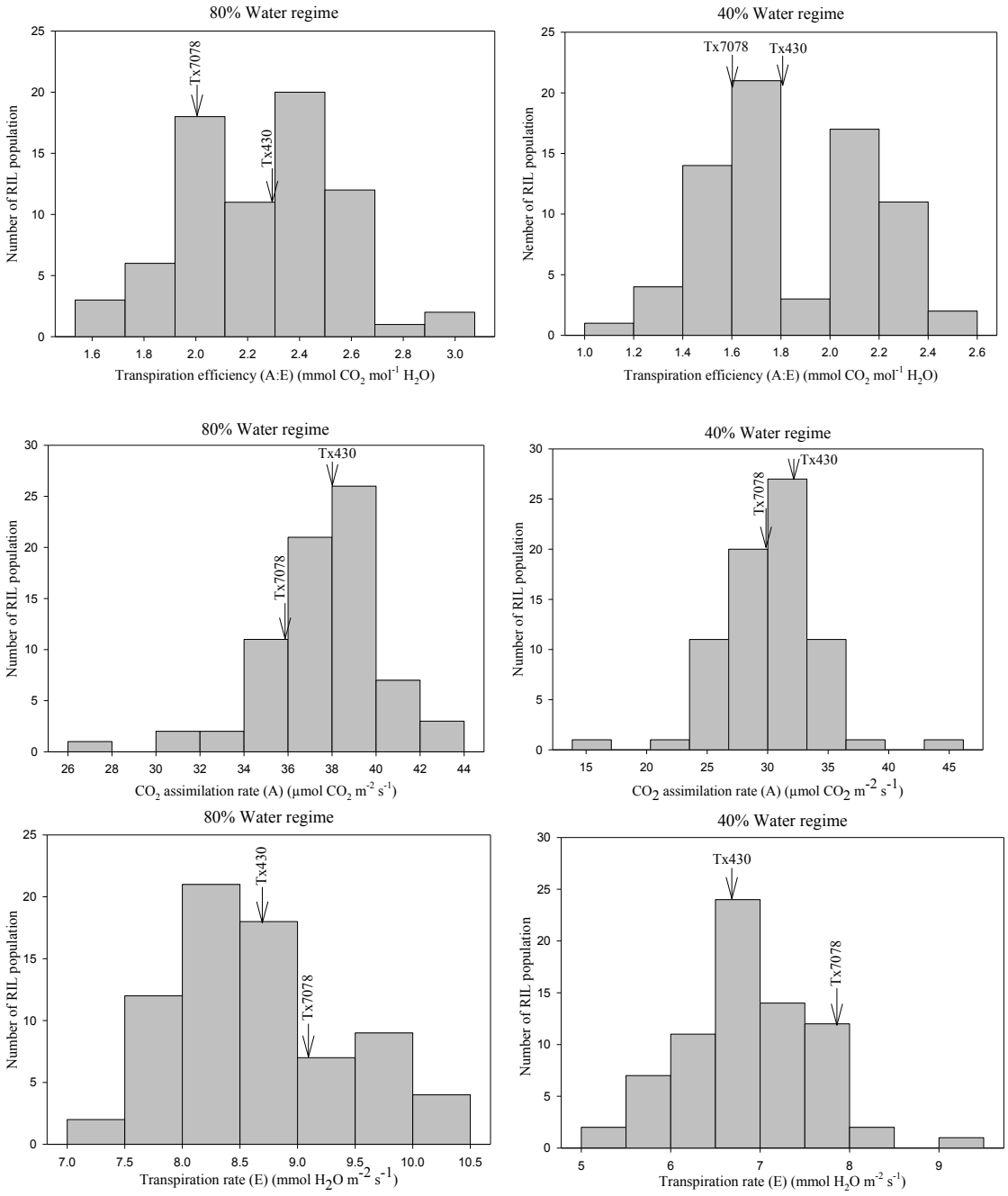

Figure 2.3. Frequency distribution of transpiration efficiency (A:E), CO_2 assimilation rate (A) and transpiration rate (E) at 80% and 40% water regime in 70 recombinant inbred lines and two parents. The mean transpiration efficiency values for Tx7078 and Tx430 are indicated by arrows.

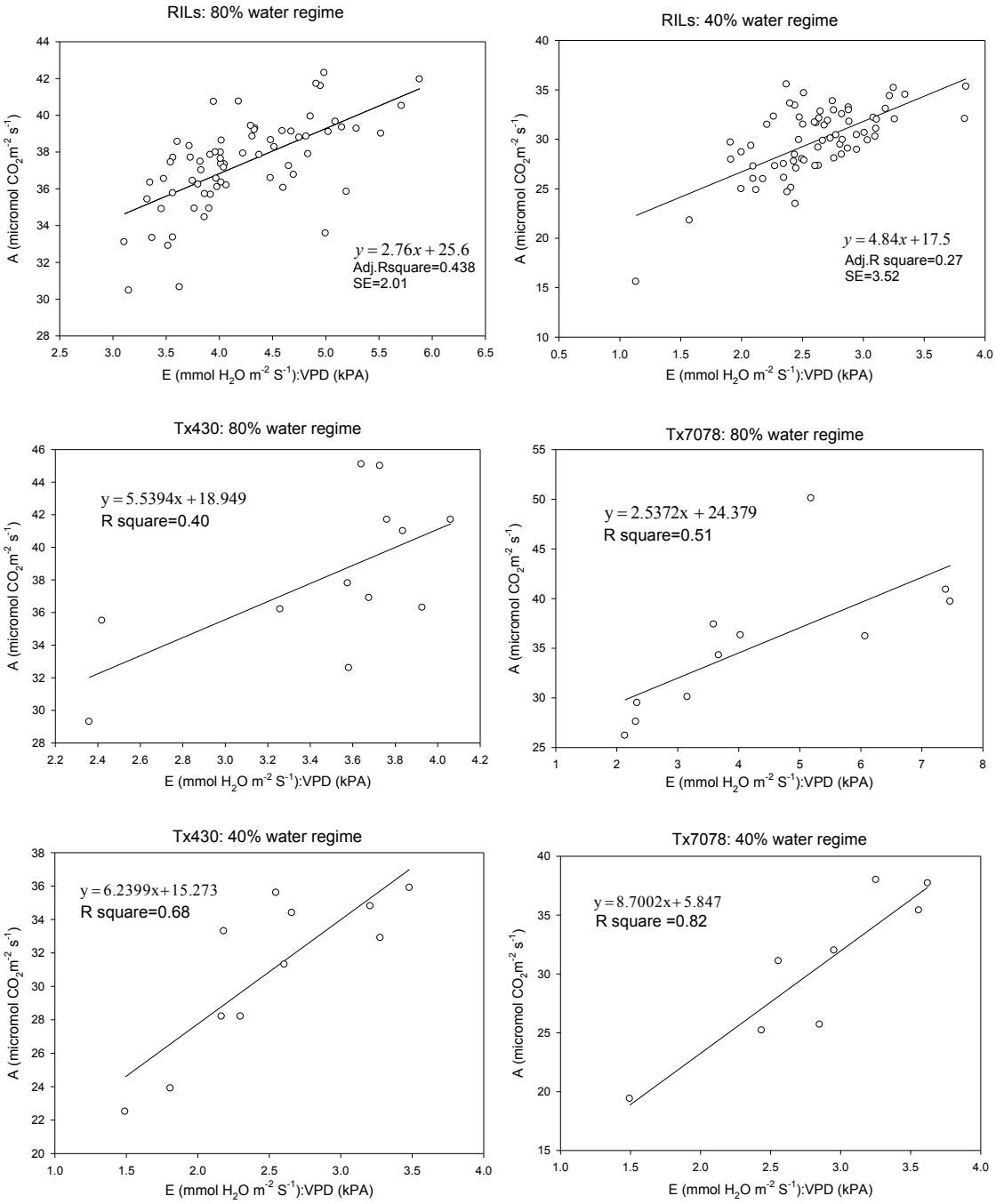

Figure 2.4. Regression of CO_2 assimilation rate (A) vs. transpiration rate (E) divided by vapor pressure deficit (EVPD) at leaf surface in 70 RILs and two parental lines at two different water regimes in greenhouse.

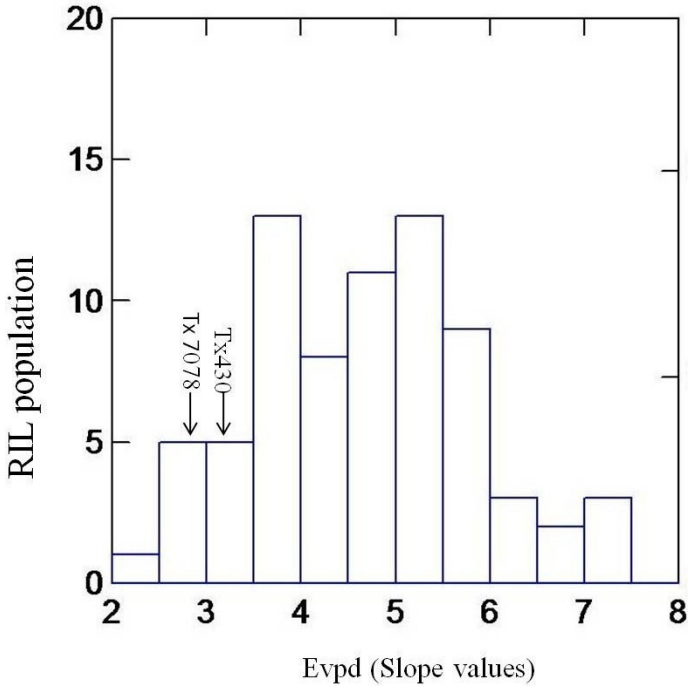

Figure 2.5. Frequency distribution graph for slope values obtained by regressing CO_2 assimilation rate (A) vs. transpiration rate (E) divided by vapour pressure deficit (vpd) in 70 recombinant inbred lines and two parents.

2.3.4 Pre-flower Whole Plant Water Use Efficiency and Biomass Relationship

Genotype did not affect cumulative transpiration per plant, total biomass or WUE except on total biomass at 40% water regime (Table 2.5). Among the RILs, Mean cumulative transpiration per plant was 0.45 kg H_2O and total biomass was 3.43 g per plant under 40% water regime.

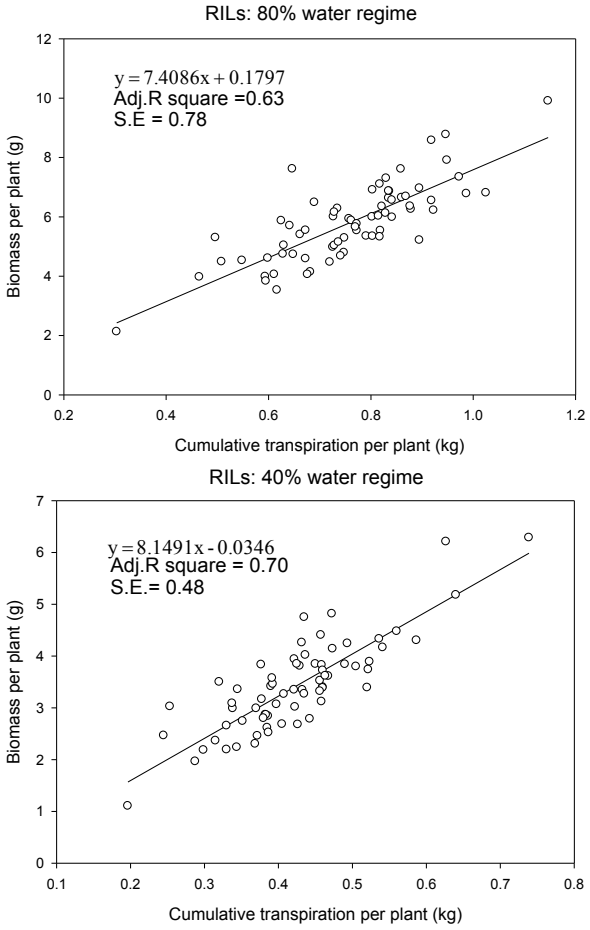

Figure 2.6. Regression of total biomass (root and shoot) per plant vs. cumulative transpiration of 70 RILs of sorghum at two different water regimes in greenhouse, Bushland, TX.

These traits increased with increased water supply from 40% to 80% water regime. Under 80% water regime mean cumulative transpiration per plant was 0.77 kg H_2O and total biomass was 5.80 g per plant (Table 2.6). However, higher water use efficiency was recorded under 40% water regime (7.89 g $kg^{-1}H_2O$) than 80% water regime (7.36 g $kg^{-1}H_2O$). Among the parents, total biomass per plant was 5.93 g for Tx430 and 6.16 g for Tx7078 under 80% water regime. In 40% water regime, total biomass per plant produced by Tx430 was 3.75 g and Tx7078 was 3.70 g (Table 2.6). Among the RILs range of whole plant water use efficiency was 5.34 to 10.34 g kg^{-1} H_2O and 5.35 to 11.02 g $kg^{-1}H_2O$ under 80% and 40 % water regime, respectively. Water use efficiency was 7.60 and 7.68 g kg^{-1} for Tx430 and 7.55 and 7.19 g kg^{-1} for Tx7078 under 80% and 40% water regime, respectively. Among the RILs, when total biomass per plant was regressed against cumulative transpiration per plant, 40% water regime had a higher slope of regression (8.14 kg) compared to 80% water regime (7.40 kg) (Figure 2.6). Slope values indicate that RILs produced ~10% more biomass per water used under 40% water regime level compared to 80% water regime.

Table 2.5. Mean squares and probability levels (*p*) from ANOVAs based on individual environment (water regimes) for whole plant water use efficiency in sorghum RIL population with four replications during 2008, Bushland, TX.

Source	df	Cumulative transpiration ($kg\ H_2O$)		Total biomass ($g\ plant^{-1}$)		WUE ($g\ kg^{-1}\ H_2O$)	
		Mean square	*p*	Mean square	*p*	Mean square	*p*
80% Water regime							
Genotype	72	0.10	>0.163	5.35	>0.440	3.08	>0.791
Replication	3	0.57	>0.000	78.08	>0.000	43.74	>0.000
Error	183(179†)	0.08		5.23		3.65	
40% water regime							
Genotype	72	0.10	>0.110	2.29	>0.009	3.82	>0.544
Replication	3	0.30	>0.010	9.01	>0.000	30.53	>0.000
Error	151	0.07		1.44		3.93	

†Error df for total biomass and WUE traits.

Table 2.6. Mean cumulative transpiration, total plant biomass, and WUE in 70 RILs sorghum lines and 2 parents grown in a green house, Bushland, Texas.

Trait	Tx430 (SD)†		Tx7078		RILs mean		RILs range	
	40% FC	80%FC	40% FC	80%FC	40% FC	80%FC	40% FC	80%FC
Cumulative transpiration (kg H_2O)	0.48 (0.06) †	0.78 (0.15)	0.49 (0.28)	0.83 (0.06)	0.45 (0.19)	0.77 (0.16)	0.19-1.44	0.30-1.49
Total biomass per plant (g)	3.75 (1.26)	5.93 (1.72)	3.70 (2.81)	6.16 (2.09)	3.43 (0.88)	5.80 (1.29)	1.10-6.28	2.13-9.90
WUE (g kg^{-1} H_2O)	7.68 (4.04)	7.60 (2.04)	7.19 (4.22)	7.55 (3.15)	7.89 (1.19)	7.36 (0.97)	5.35-11.02	5.34-10.34

†Standard deviation in parenthesis.

2.3.5 Trait Correlation

Pearson correlation coefficients indicate that transpiration efficiency was significantly correlated (0.82 and 0.61 under 40% and 80% water regimes, respectively) with CO_2 assimilation rate than EVPD (Table 2.8). However, CO_2 assimilation rate and EVPD were found to be significantly correlated with 0.53 and 0.66 under 40% and 80% water regimes, respectively. Whole plant water use efficiency was significantly correlated with total biomass per plant (0.56 and 0.55 under 80 % and 40% water regimes, respectively). The strong positive correlation among transpiration ratio with A and WUE (Table 2.7) with total biomass production indicate that genotypic differences in transpiration efficiency were associated with genotypic variability in both CO_2 assimilation rate and biomass production. There was no strong correlation observed between leaf level transpiration ratio and whole plant water use efficiency among the 70 RILs but the trend was generally positive (*r* of 0.13 and 0.29 under 80% and 40% water regimes). This might be due to the fact that transpiration efficiency based on leaf level gas exchange measurements will not account for root and/or dark respiration (Peng and Krieg 1992). In addition, due to experimental error and variation in climatic condition such as light intensity and ambient CO_2 concentration over the growing season might have resulted in inconsistent data on whole plant water use efficiency. However, Peng et al. (1991) suggested that leaf photosynthetic rate measured prior to flowering is a good indicator of productivity of grain sorghum.

2.4 Conclusions

These results provide further evidence that there is genetic variability among genotypes for gas exchange rates (A, E and A:E) at pre-flowering in sorghum with heritability values that suggest scope for improved WUE and productivity. High correlation between the transpiration ratio vs. CO_2 assimilation rates and whole plant WUE vs. total biomass per plant were similar to the results reported by Balota et al. (2008) and other cited studies of Krieg and his colleagues. We believe that our results are potentially useful to develop the genetic map and identify the genes involved in pre-flower drought tolerance, particularly at the GS2 stage, and increased sorghum production under U.S. Great Plains environments, Africa and parts of India. Because of the difficulties and costs associated with phenotyping these traits, genetic analysis and characterization of QTL associated with the traits are absolutely essential if selection is to be practiced for them.

Table 2.7. Correlation coefficients (r) for gas exchange traits and whole plant water use efficiency traits of 70 RILs of sorghum genotypes in the greenhouse.

	Transpiration (EVPD)	Transpiration ratio	Cumulative transpiration	Total biomass	WUE
80% water regime					
CO_2 Assimilation rate	0.67***	0.61***	-0.10	-0.03	0.09
Transpiration (E/vpd)		0.30**	-0.05	-0.08	-0.08
Transpiration ratio			0.08	0.16	0.13
Cumulative transpiration				0.64***	-0.01
Total biomass					0.57***
40% water regime					
CO_2 Assimilation rate	0.53***	0.82***	-0.12	-0.01	0.27
Transpiration (E/vpd)		0.16	-0.13	-0.12	-0.06
Transpiration ratio			-0.02	0.05	0.29
Cumulative transpiration				0.48***	-0.14
Total biomass					0.55***

**Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

CHAPTER III

IDENTIFICATION OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH TRANSPIRATION EFFICIENCY RELATED TO PRE-FLOWER DROUGHT TOLERANCE IN SORGHUM

3.1 Introduction

Drought is the most important abiotic stress factor in agriculture limiting crop productivity in arid and semi-arid regions of the world (Boyer, 1982). Current research in both public and private sector breeding programs across most crop species almost always place some emphasis on improving drought tolerance. Understanding the genetic basis of how plants respond to moisture stress should provide an opportunity to improve drought resistance. Even though considerable work has been done on sorghum response to moisture stress (Tuinstra et al., 1996; Xu et al., 2000; Kebede et al., 2001; Sanchez et al., 2002; Haussmann et al., 2002; Borrell et al., 2004; Kassahun et al., 2010), there has been little emphasis on the use of specific physiological traits to enhance drought stress tolerance. This is usually because these reactions are complex, and they are dependent on many factors, which include but are not limited to the climate, the timing and the severity of moisture stress. Consequently, drought tolerance is one of the most difficult traits to improve in breeding programs. One way to reduce complexity in a stress such as drought tolerance is to look at specific physiological traits which can be easily measured and quantified. Physiological traits like transpiration efficiency, which is defined as change in CO_2 assimilation rate (A) per unit change in transpiration rate (E), can be one important way to increase the water productivity under drought stress conditions.

Sorghum is the world's fifth most important grain crop based on tonnage, after maize (*Zea mays* L.), wheat (*Triticum aestivum*), rice (*Oryza sativa*), and barley (*Hordeum vulgare*) (www.fao.org), and provides staple food for millions of people in semi-arid tropics of Africa and Asia. In 2009, sorghum was produced on approximately 40.23 million hectares with an average yield of 1.49 metric tons per hectare worldwide (USDA, 2010). Sorghum is one of the most drought tolerant (Blum, 2004) and water efficient cereal grains and it is well adapted to semiarid tropical and subtropical environments (Rooney, 2004). The crop is an excellent model plant species in which to evaluate cereal crop drought adaption mechanisms (Rosenow et al., 1983).

Sorghum has a highly diverse source of useful alleles which offers the potential to breed for even more drought tolerant and water efficient genotypes (Rosenow and Dahlberg, 2000). Sorghum lines with a distinct phenotypic response to pre-flowering and post-flowering moisture stress have been described and characterized. Excellent sources of resistant to each source of resistance are available (Rosenow, 1993; Balota et al., 2008). Pre-flowering and post-flowering drought resistances are also distinctly different among genotypes and very likely controlled by different genetic mechanisms (Rosenow, 1987). In sorghum, pre-flowering drought stress occurs when plants are under severe moisture stress prior to flowering, especially from panicle differentiation until flowering. Important symptoms include leaf rolling and erectness delayed flowering and reduced height. Post-flowering drought stress (also referred to as "terminal drought") causes premature leaf senescence and can lead to stalk lodging, stalk rot disease, and significant yield loss (Rosenow and Clark, 1995).

Many studies have shown that sorghum possesses genetic variation for many traits related to drought tolerance (Krieg and Hutmacher, 1986; Kidambi et al., 1990a, Peng et al., 1991; Peng and Krieg, 1992; Krieg et al., 1992, Balota et al., 2008). However, selection for drought tolerance while maintaining high productivity has been a challenge (Rosenow et al., 1983), in part due to the difficulty of quantifying drought and the relative absence of a genetic basis for specific trait associated with drought tolerance. Drought resistance in sorghum is a complex trait affected by several interacting plant and environmental factors which makes this trait difficult, time consuming or expensive to study using traditional genetic and physiological methods. Molecular markers allow breeders to track the specific genetic loci that respond to moisture stress tolerance without extensive field trials (Tanksley, 1993), and to focus on the function of each locus without the confounding effect of segregating loci (Yang et al., 1993). Therefore, use of molecular markers and QTL analysis of drought tolerance might lead to better understanding of this trait. If successful, this will reduce the time and cost involved in field trials, increase the breeding efficiency as defined as gain per year and allow breeder to select simultaneously for drought tolerance and other agronomic traits. Now that diverse parents have been identified, genetic mapping of quantitative trait loci (QTL) for transpiration efficiency (change in CO_2 assimilation rate (A) per unit change in transpiration rate (E)) related to pre-flower drought tolerance is an important step towards developing pre-flowering drought-resistant hybrids in sorghum.

Several studies have characterized and mapped post-flowering drought tolerance and the stay green trait using various sorghum populations (Tunistra et al., 1997; Crasta

et al., 1999; Subudhi et al., 2000; Xu et al., 2000; Harris et al., 2007). Tunistra et al. (1997) identified 13 regions of the genome associated with one or more measures of post flowering drought tolerance using 98 RILs in sorghum developed from cross between Tx7078 X B35. Two QTL were found to have major effect on yield and stay green under post flowering drought. Crasta et al., (1999) identified three major and four minor stay green QTLs and two maturity QTLs using a set of RILs obtained from the cross B35 X Tx430 in sorghum. They observed that these stay green QTLs were completely independent of QTLs influencing maturity. Xu et al., (2000) identified four stay green QTLs located on the three linkage groups A (chromosome 1), D (chromosome 4) and J (chromosome 10) along with three QTLs for chlorophyll content. These explained 25-30% of the phenotypic variability under post-flowering drought stress using F_7 RILs derived from the cross of B35 X Tx7000. In Australia, Tao et al., (2000) identified three regions associated with stay green in multiple environment trials using 160 RILs derived from cross between QL39 X QL 41 in sorghum using 17 SSR and 101 RFLP markers. Harris et al. (2007) mapped the post-flowering stay green trait to four major QTLs (*Stg1-Stg4)* using a population derived from BTx642 X RTx7000 (BTx642 is the released name of B35).

Pre-flowering (defined as the period from panicle differentiation to anthesis) drought adaptation is different and important because it is during this stage that stand, tiller number, panicle size, grain number and grain yield are determined (Sanchez, 2002; Squire, 1993). In the US Great Plains and elsewhere, water stress occurs most frequently during this sensitive stage due to low precipitation, low humidity and high evaporative

demand (Bandaru et al., 2006). Tuinstra et al.(1996) identified six regions in the sorghum genome associated with pre-flowering drought tolerance using recombinant inbred lines obtained from cross of Tx7078 X B35, which are resistant and susceptible, respectively, to pre-flowering drought.

Given the paucity of information on pre-flowering drought-stress, this study was undertaken to identify the QTL that condition traits associated with pre-flowering drought stress using a RIL population derived from the cross Tx430 x Tx7078.

3.2 Material and Methods

3.2.1 Genetic Map Construction

Seventy F_6 RILs derived from a cross between Tx430 x Tx7078 and the two parental lines were planted in the green house (Biotechnology Center for Crop Improvement, College Station, TX) on 15, April 2009. DNA was extracted from the leaf tissue of seedlings at 12 days after emergence using FastDNA® Spin Kit (MP Biomedical, LLC, France) extraction procedure. DNA concentration was estimated by using Qubit® Flurometer (Invitrogen™, Turner Biosystems). Genotyping was performed using the digital genotyping method that collects information on polymorphic sequences from specific sites across the sorghum genome using Illumina GAII sequencer. The Illumina GAII is a high throughput DNA sequencer capable of sequencing ~200M templates per run with sequence read lengths of ~38 bp (or more). Initially a total of 403 DGA (Digital Geotyping Analysis) indel / single-nucleotide polymorphism (SNP's) marker data were obtained from 3X cluster analysis for 70 RILs (Sequencing by Dr. Daryl Morishige and data analyzed by Dr. Patricia Klein, Texas A&M University). Mapmaker/Exp version

3.0b (Whitehead Institute, Cambridge, MA), Kosambi centiMorgan (cM) function, was used to create the genetic map. The 'ri self' setting was used and missing, non-parental, and heterozygous alleles were treated as missing data for map construction and QTL mapping. Linkage groups were assigned to chromosomes using the designation of Kim et al., (2005) and displayed in the orientation of Menz et al., (2002). After obtaining the preliminary genetic map, a final map was obtained by deleting the non-informative markers with final total of 261 informative markers on 10 chromosomes. A segregation distortion test was performed using the AntMap version 1.1 (Iwata and Ninomiya, 2006).

3.2.2 QTL Analysis

QTLs were identified by performing the composite interval mapping with Windows QTL Cartographer version 2.5 (WINQTL) (Wang et al., 2007). We used WINQTL settings RI1 for the cross type and 2 cM for the walk speed. Standard regression analysis was performed with a 0.1 in/out probability and with window size of 10 cM. Permutation threshold at a 0.05 significance level was obtained for each trait using 1000 permutations. The QTL Figures were created using the MapChart 2.2 (Voorrips, R.E., 2002).

Genetic variation for the transpiration efficiency was estimated using the same population. Phenotypic measurements for transpiration efficiency, data analysis and results were presented in Chapter II. The data was averaged over four replications for QTL analysis. Individual replicated phenotypic data were also used to obtain further in-depth information on the QTLs responsible for transpiration efficiency traits.

3.3 Results

3.3.1 Linkage Map

The genetic map (Figure 3.1) of RILs had a total length of 1128.6 centiMorgans (cM in the kosambi function), with 261 addition and deletion informative polymorphic markers distributed over 10 linkage groups. The average distance between the adjacent markers was 4.3 cM. The numbers of markers per chromosome (SBI) ranged from 11 (SBI-05) to 45 (SBI-01), with an average of 26 markers per chromosome. SBI-01 and SBI-10 were the longest linkage groups, while the SBI-05 and SBI-08 were the shortest. Approximately 91% of the intervals between adjacent markers were smaller than 10 cM and 7% were in the range of 10 to 20 cM and only 3 markers were observed in the range of 30 to 35 cM.

On average, 50% of the genome was homozygous for Tx430 alleles, 43.5% of the genome was homozygous for Tx7078 alleles, and 5.5% of the genome was heterogygous. The chi-square test of frequencies of individual parental alleles in the F_6 population indicated that 68 of 261 mapped marker loci (26%) displayed significant deviation from the expected 1:1 ratio. Out of 68 segregation distorted markers, 21 markers were observed on SBI-02. The moderate amount of segregation distortion found in this mapping population should not greatly affect the QTL analysis, and no additional steps were made to take this distortion in account. In most cases, allele frequency

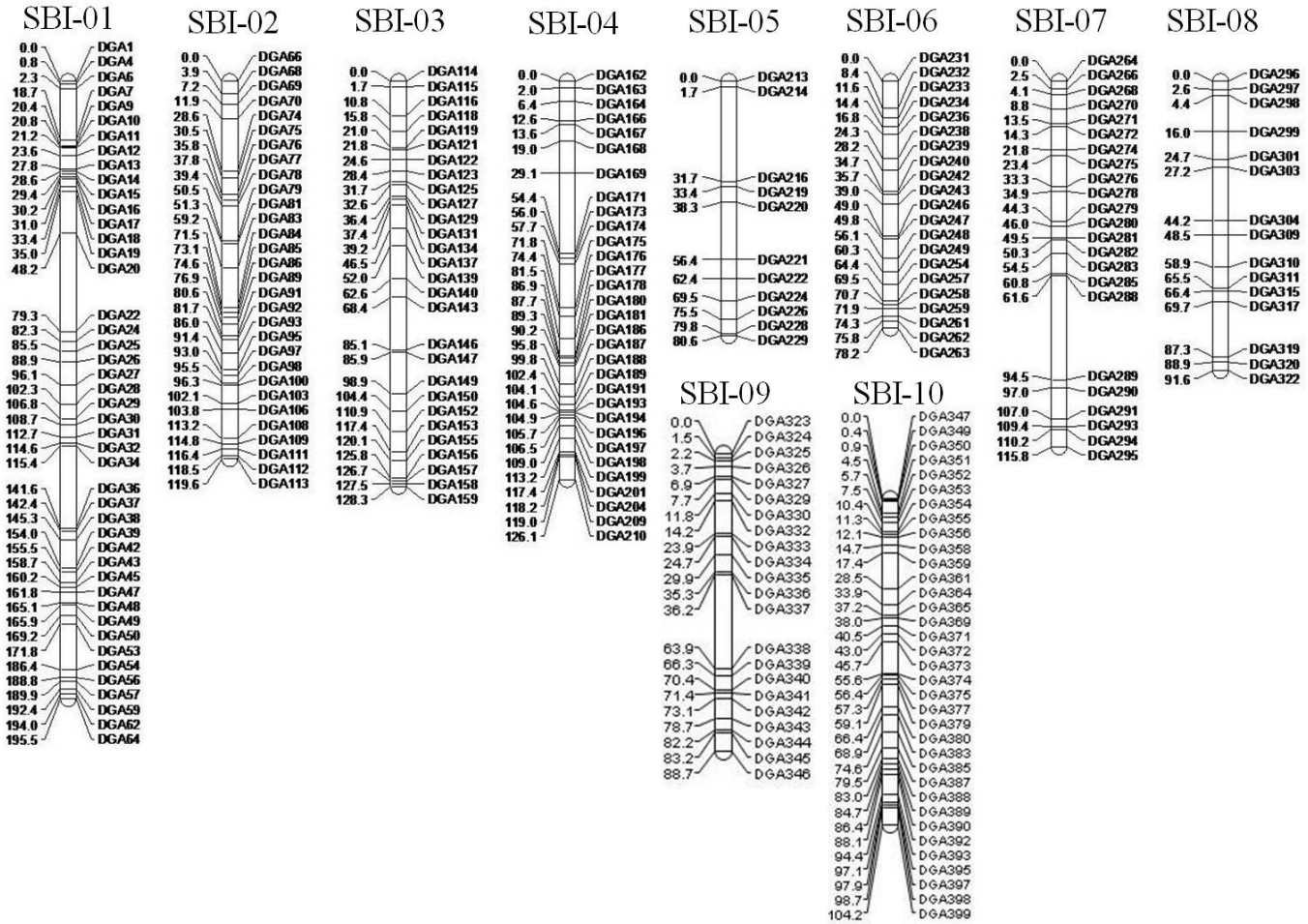

Figure 3.1.Genetic map derived from Tx430 x Tx7078 recombinant inbred line (RIL) of sorghum population. The 10 sorghum chromosomes are displayed in the orientation of Kim et al., (2005). Markers names DGA represent Digital Genome Analyzer (GA-II).

distortion favored the Tx430 allele, which implies that Tx430 alleles are associated with the increased fitness.

3.3.2 QTL Associated with Transpiration Efficiency

Three significant QTL (LOD > 3.9) for transpiration efficiency were identified on SBI-09 and SBI-10 (Table 3.1 and Figure 3.2) under 80% water regime with one LOD interval length ranging from 5.3 cM to 5.7 cM. The two QTL on SBI-09 accounted for 19% and 21% and QTL on SBI-10 accounted for 17% of the total phenotypic variation for the transpiration efficiency trait. Combined, these three QTLs explained 57% of the total phenotypic variation for transpiration efficiency with a cumulative LOD score of 12.9. In the transpiration efficiency QTL on SBI-10 (TE_80%.3), alleles from Tx430 improved the transpiration efficiency trait. Interestingly, no QTL for TE were detected at the 40% water regime.

Two QTL (A_40% and A_80%) influencing CO_2 assimilation rate were identified on SBI-05 and SBI-01. These accounted for 15 and 12 % of the phenotypic variability, respectively with the cumulative peak LOD score of 6.1. In both QTLs, alleles from Tx430 contributed to improvement in the CO_2 assimilation rate.

Two QTL (E_40% and E_80%) were detected for transpiration rate on SBI-01 and SBI-07. These QTL were detectable in both the 40% and 80% water regimes (Table 3.1). These two QTL together accounted for 25% of the phenotypic variability with LOD scores of 2.6 and 3.1 for E_40% QTL and E_80% QTL, respectively. In both QTL, alleles from Tx430 contributed to an increase in transpiration rate.

Table 3.1. Composite interval mapping of QTLs influencing transpiration efficiency, CO_2 assimilation rate and transpiration rate related to pre-flowering drought stress in grain sorghum F_6 RILs from the cross of Tx430 x Tx7078.

Traits	Environment	QTLs	SBI #	Flanking Markers	1- LOD Interval length (cM)	QTL position (cM)	Peak LOD score	Additive effect	Increased effect	R^2
Transpiration efficiency (A:E)	TE80%Ave	TE_80%. 1	9	DGA326-DGA327	5.7	5.7	4.0	-0.14	Tx7078	0.19
	TE80%Ave	TE_80%. 2	9	DGA329-DGA330	5.5	11.7	4.7	-0.15	Tx7078	0.21
	TE80%Ave	TE_80%. 3	10	DGA395-DGA398	5.3	97.1	4.2	0.14	Tx430	0.17
CO_2 Assimilation rate (A)	40%Ave	A_40%	5	DGA216-DGA219	16.8	31.7	3.3	1.67	Tx430	0.15
	80%Ave	A_80%	1	DGA18-DGA20	22.3	48.2	2.9	0.98	Tx430	0.12
Transpiration rate (E)	40%Ave	E_40%	1	DGA29-DGA30	9.7	104.3	2.6	0.25	Tx430	0.12
	80%Ave	E_80%	7	DGA291-DGA293	13.7	107	3.1	0.28	Tx430	0.13

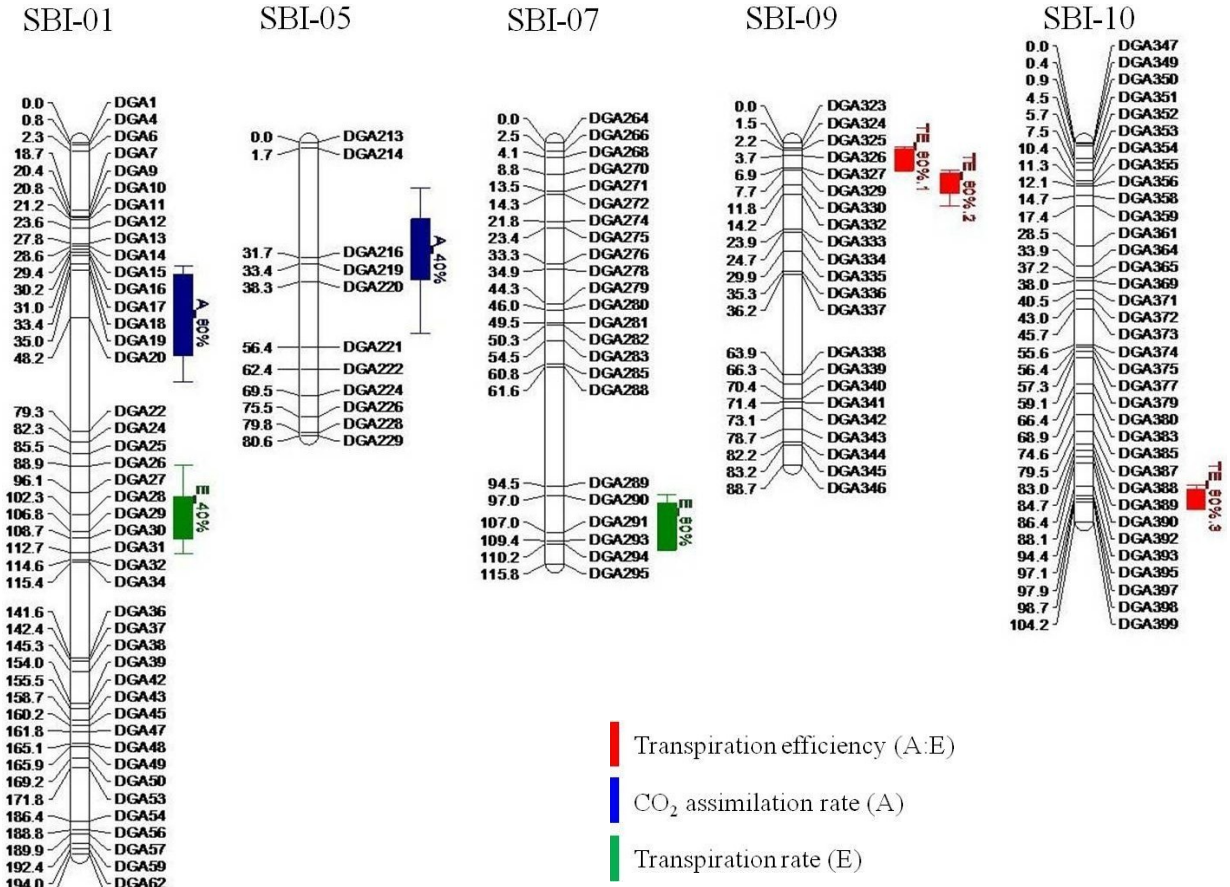

Figure 3.2. Digital Genotyping (GA-II) linkage map of sorghum showing positions of quantitative trait loci (QTLs) influencing transpiration efficiency, CO_2 assimilation rate and transpiration rate under drought stress environment. The map was developed using the F_6 RIL population of the cross Tx430 x Tx7078.

3.3.3 Mapping of Transpiration Efficiency Traits Based on Replication

Based on analysis of individual replicated phenotypic data, six transpiration efficiency QTLs were identified on SBI-01, SBI-02, SBI-06 and SBI-09 under 40% water regime and two QTLs were detected on SBI-03 and SBI-06 under 80% water regime (Table 3.2 and Figure 3.3). These eight QTLs explained phenotypic variability for transpiration efficiency ranging from 14 to 27% with a cumulative LOD score of 46. Alleles from Tx430 contributed favorably for two QTL (TE_40%_2 and TE_80%_2); the remainder of favorable alleles at all other loci were from Tx7078.

Four QTLs (A_40%_1, 2, 3, and 4) influencing the CO_2 assimilation rate were identified on SBI-08, SBI-09 and SBI-10 under 40% water regime. Under the 80% water regime, six QTLs were detected on SBI-01, SBI-02, SBI-03 and SBI-10 for CO_2 assimilation rate among four replications. These QTLs contributed to the phenotypic variability for CO_2 assimilation rate ranging from 11 to 24% with peak LOD score ranging from 2.7 to 5.1.

Three QTLs (E_40%_1, 2, 3 and 4) were detected under 40% water regime on SBI-01, SBI-02 & SBI-03 and nine QTLs (E_80%_1, 2, 3, 4, 5, 6, 7, 8, and 9) under 80% water regime on SBI-01, SBI-02, SBI-03, SBI-04, SBI-07, SBI-08 and SBI-10 were associated with transpiration rate. These QTLs contributed phenotypic variability for transpiration rate ranging from 13 to 38% with LOD score ranging from 3.0 to 7.4.

Table 3.2. Composite interval mapping of QTLs influencing transpiration efficiency, CO_2 assimilation rate and transpiration rate related to pre-flowering drought stress in grain sorghum F_6 RILs from the cross of Tx430 x Tx7078 based on replication.

Trait	Environment	QTLs	SBI #	Flanking makers	1 LOD Interval length (cM)	QTL position (cM)	Peak LOD score	Additive effect	Increased effect	R^2
Transpiration efficiency (A:E)	40%_Rep3	TE_40%_1	1	DGA32-DGA34	11.0	114.6	3.5	-0.08	Tx7078	0.15
	40%_Rep3	TE_40%_2	2	DGA111-DGA112	8.0	116.4	3.6	0.08	Tx430	0.16
	40%_Rep4	TE_40%_3	2	DGA79-DGA81	13.0	50.5	2.8	-0.12	Tx7078	0.19
	40%_Rep1	TE_40%_4	9	DGA327-DGA329	5.3	7.7	5.6	-0.09	Tx7078	0.26
	40%_Rep1	TE_40%_5	9	DGA330-DGA332	7.2	14.2	3.9	-0.08	Tx7078	0.19
	80%_Rep1	TE_80%_1	3	DGA152-DAG153	12.3	114.9	4.0	-0.08	Tx7078	0.21
	80%_Rep4	TE_80%_2	6	DGA259-DGA261	18.3	71.9	2.5	0.15	Tx430	0.14
	80%_Rep2	TE_40%_3	6	DGA247-DGA248	7.9	53.8	5.0	-0.10	Tx7078	0.28
CO_2 Assimilation rate (A)	40%_Rep4	A_40%_1	8	DGA311-DGA315	6.1	66.4	4.0	-2.10	Tx7078	0.22
	40%_Rep4	A_40%_2	8	DGA317-DGA319	16.2	73.7	3.0	-2.09	Tx7078	0.22
	40%_Rep1	A_40%_3	9	DGA330-DGA332	9.5	13.8	4.4	-2.15	Tx7078	0.20
	40%_Rep4	A_40%_4	10	DGA397-DGA398	7.4	98.7	3.5	1.63	Tx430	0.11
	80%_Rep3	A_80%_1	1	DGA7-DGA9	4.7	18.7	3.1	-2.36	Tx7078	0.13
	80%_Rep3	A_80%_2	1	DGA37-DGA38	18.2	142.4	2.8	1.87	Tx430	0.12
	80%_Rep4	A_80%_3	2	DGA78-DGA79	13.1	41.4	5.1	2.32	Tx430	0.24
	80%_Rep1	A_80%_4	3	DGA152-DGA153	5.4	114.9	3.3	-2.10	Tx7078	0.17
	80%_Rep4	A_80%_5	3	DGA129-DGA131	11.0	36.4	3.2	1.56	Tx430	0.13
	80%_Rep3	A_80%_6	10	DGA392-DGA393	8.3	90.1	4.0	2.40	Tx430	0.19
Transpiration rate (E)	40%_Rep3	E_40%_1	1	DGA28-DGA29	4.5	102.1	7.1	1.14	Tx430	0.38
	40%_Rep3	E_40%_2	1	DGA32-DGA34	6.5	114.6	4.5	-0.83	Tx7078	0.22
	40%_Rep4	E_40%_3	2	DGA103-DGA106	11.5	102.1	4.1	0.45	Tx430	0.26
	40%_Rep3	E_40%_4	3	DGA137-DGA139	12.3	52.0	3.0	0.49	Tx430	0.14
	80%_Rep3	E_80%_1	1	DGA45-DGA47	4.7	160.2	4.3	0.43	Tx430	0.18
	80%_Rep4	E_80%_2	2	DGA77-DGA78	14.0	37.8	7.5	0.56	Tx430	0.27
	80%_Rep2	E_80%_3	2	DGA69-DGA70	17.0	11.9	3.0	-0.53	Tx7078	0.12
	80%_Rep4	E_80%_4	3	DGA129-DGA131	3.7	36.4	4.5	0.41	Tx430	0.18
	80%_Rep3	E_80%_5	4	DGA189-DGA191	4.6	102.4	3.4	-0.39	Tx7078	0.15
	80%_Rep2	E_80%_6	7	DGA291-DGA293	14.4	107	3.5	0.55	Tx430	0.15
	80%_Rep4	E_80%_7	7	DGA274-DGA275	9.6	23.4	3.7	0.37	Tx430	0.14
	80%_Rep1	E_80%_8	8	DGA304-DGA309	9.2	69.7	3.9	0.47	Tx430	0.16
	80%_Rep4	E_80%_9	10	DGA395-DGA397	9.0	97.9	3.8	0.42	Tx430	0.14

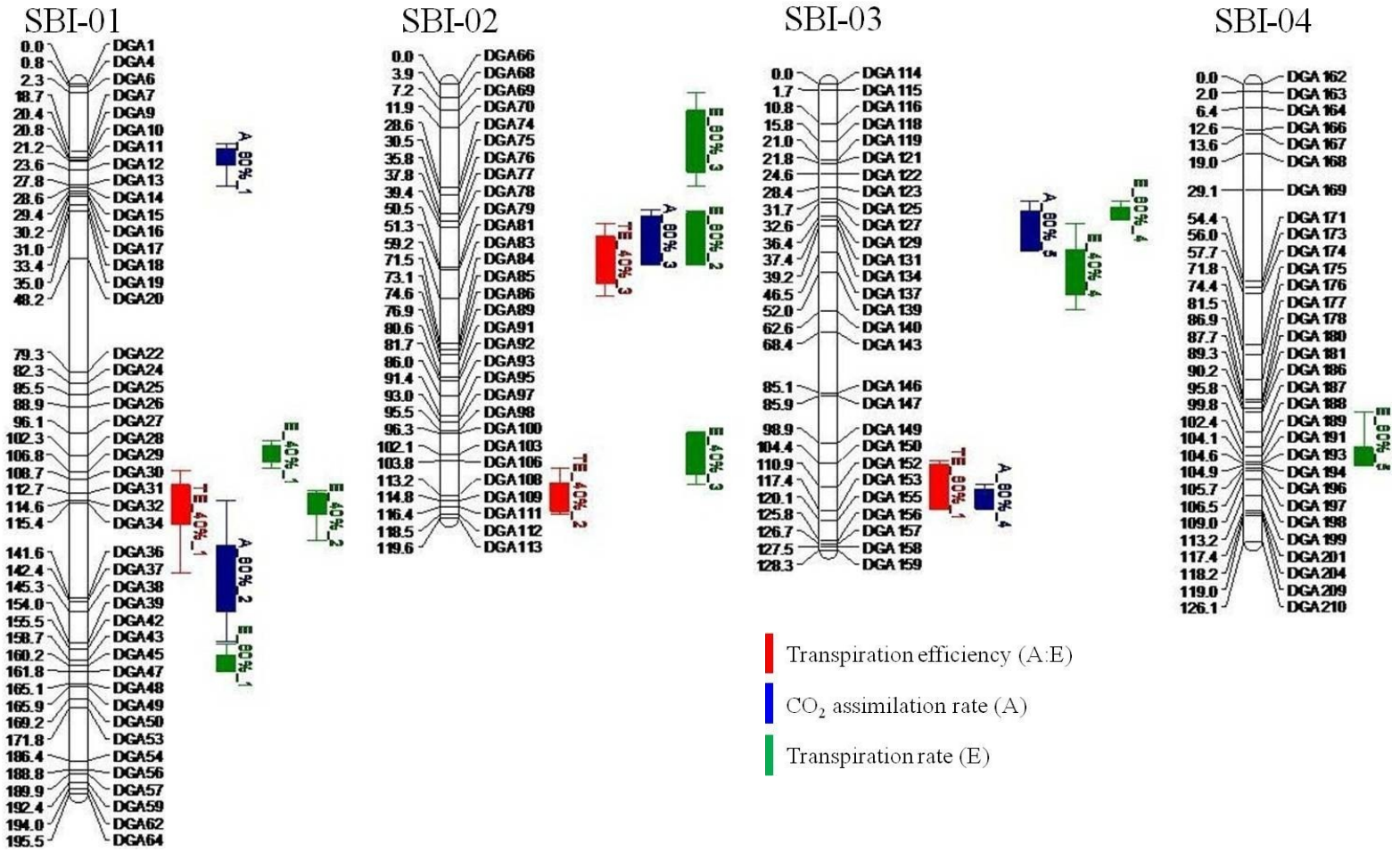

Figure 3.3. Digital Genotyping (GA-II) linkage map of sorghum showing positions of quantitative trait loci (QTLs) influencing transpiration efficiency, CO_2 assimilation rate and transpiration rate under drought stress environment based on replication data. The map was developed using the F_6 RIL population of the cross Tx430 x Tx7078.

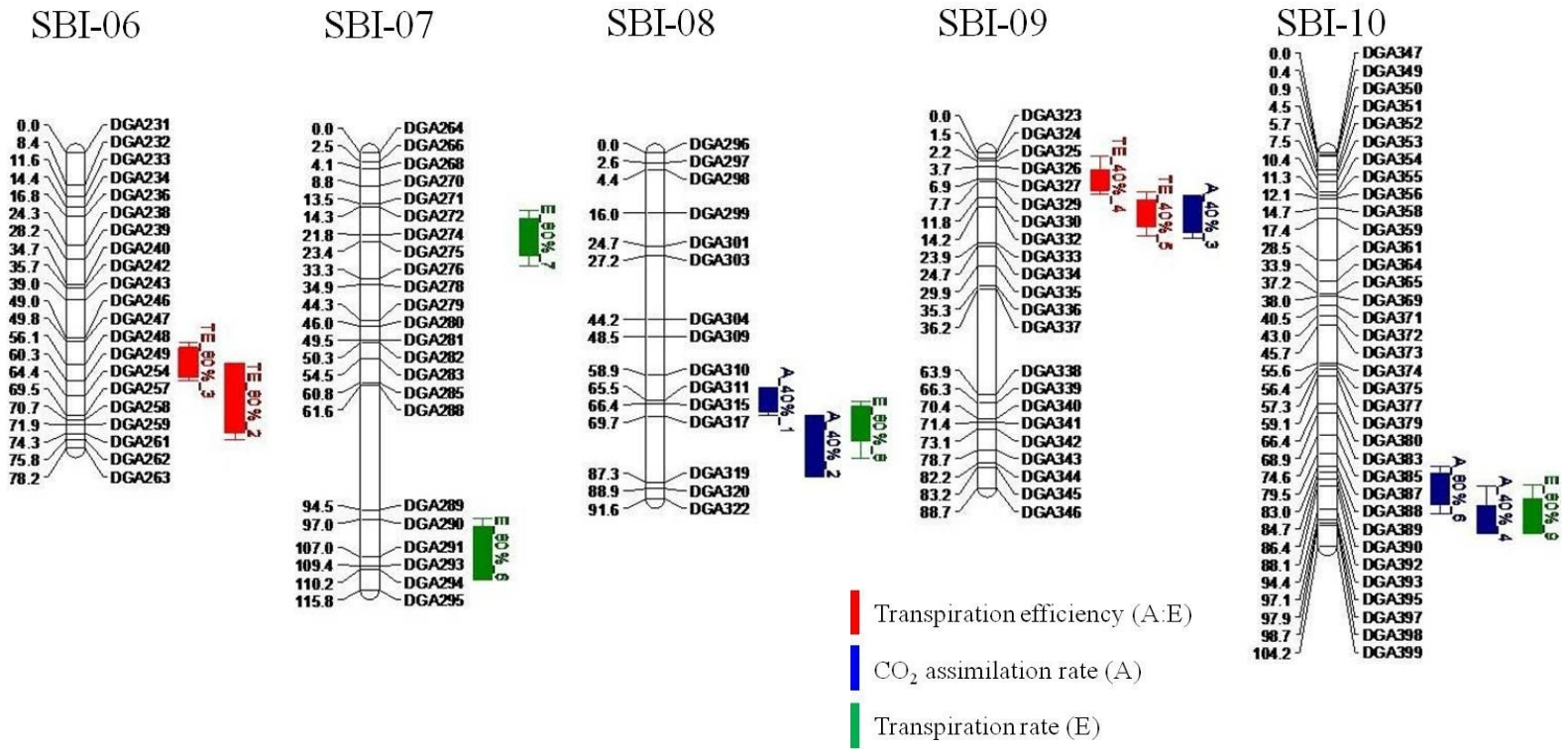

Figure 3.3. Continued

3.4 Discussion and Conclusion

The goal of this project is to understand the genetic basis of the sorghum transpiration efficiency trait and to identify the genes/loci that contribute to this trait in sorghum genotypes. Herein we evaluated a RIL population derived from two contrasting parents for transpiration efficiency related to pre-flower drought tolerance. Tx430 has a higher A:E ratio and Tx7078 has a lower A:E ratio based on previous experiments (Balota et al., 2008). Therefore, this population was structured to find major QTL for higher transpiration efficiency. Two other studies have identified QTL for pre-flower drought tolerance in sorghum. Tuinstra et al. (1996) identified the six regions of the genome specifically associated with pre-flowering drought tolerance in sorghum either with yield per se or stability of yield, seed set or height on linkage groups D, F and M using RIL obtained from a cross between Tx7078 X B35, which are tolerant and susceptible parents to pre-flower drought, respectively. These regions were not detected under fully irrigated conditions, indicating that these QTLs were only expressed under drought stress. They also found strong association between the QTLs associated with seed set stability with early maturity which reflect the pleiotropic effect of maturity on seed set. Kebede et al. (2001) found four QTLs associated with pre-flowering drought-stress tolerance on linkage group C, E, F and G based on the pre-flower stress ratings using F_7 recombinant inbred line population derived from the cross SC56 X Tx7000. One major QTL for pre-flowering drought stress, *pfr* G, was consistently detected in two environments and explained 15- 37% of the phenotypic variation. This region is very important since it co-localized with QTL for other traits such as stay green, lodging

resistance, flowering time and plant height. Another pre-flowering drought tolerant QTL, *pfr F,* which accounted for 22-25% of the phenotypic variation, was detected consistently over two environments. This genomic region also overlaps with flowering time. Rosenow et al. (1996) indicated that this association was probably a result of the effect of pre-flowering drought stress on flowering time. Since there are differences in populations, locations and type of measurements made in these QTL studies and the current study, there was no co-localization noticed between these QTL studies. The current study considered the specific physiological trait (transpiration efficiency) before flowering to identify the loci responsible for drought tolerance. To date there have been no published molecular genetics study on transpiration efficiency trait related to pre-flower drought tolerance in sorghum.

In the present study, we identified three QTLs associated with transpiration efficiency trait, two on SBI-09 and one on SBI-10 with one LOD interval length ranging from 5.3 to 5.7 cM, which accounted for 17% - 21% of the phenotypic variation. This gives an opportunity to look within this interval length of 5.3 Mbp genomic regions to explore whether specific genes present in these region which are already associated with specific physiological mechanisms. If so, we can utilize this information for further selection process in the breeding program. Since, the map location of QTLs for transpiration efficiency was based on data for only one year (albeit under controlled conditions), the utility of the loci identified will depend on the level of expression in multiple environments and different genetic backgrounds. However, to our knowledge

this is the first report on mapping of QTLs influencing transpiration efficiency related to pre-flower drought tolerance in sorghum.

Improving drought tolerance is always challenging because drought-stress is unpredictable and strongly influenced by timing and intensity during different stages of crop growth. Rosenow and Clark (1981) reported that sorghum genotypes that are drought tolerant during one growth stage are often susceptible at other times. With respect to post-flowering drought tolerance, there are several studies (Tunistra et al., 1997; Crasta et al., 1999; Subudhi et al., 2000; Xu et al., 2000; Kebede et al., 2001; Harris et al., 2007) that have characterized and mapped the stay green trait using various sorghum populations. Kebede et al. (2001) reported that stay green QTLs (*Stg A, Stg G* and *Stg J)* were found to be consistent across the sorghum population and different environments. Therefore, incorporation of genes for both stay green and pre-flower drought tolerance is likely to improve the future sorghum cultivars to withstand drought at different stages of the crop growth. In this study, QTLs identified for transpiration efficiency traits related to pre-flower drought tolerance were highly significant and have independent location for genomic regions on SBI-09 and SBI-10. Therefore, the pyramiding of favorable alleles for transpiration efficiency trait and stay green together through marker assisted selection may help in breeding to develop the sorghum cultivars that are more tolerant to both pre-flower and post-flower drought.

CHAPTER IV

CO-LOCALIZATION OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH TRANSPIRATION EFFICIENCY AND AGRONOMIC TRAITS RELATED TO PRE-FLOWER DROUGHT TOLERANCE IN SORGHUM

4.1 Introduction

Worldwide during 2009, sorghum is harvested in an area of 43.7 m ha with average yield of 1.41 metric tons per hectare. Thus, in terms of world production, sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop after wheat, maize, rice and barley (FAO, 2009) and it is a staple food crop for 500 to 700 million people in arid and semiarid regions primarily of Africa and some of Asia. In terms of utilization, worldwide, almost half of the grain sorghum produced is used as animal feed. More recently sorghum has been proposed as a dedicated cellulosic bioenergy feedstock (Rooney et al., 2007).

Sorghum is known for its extensive phenotypic and genotypic variation in response to drought (Blum, 1979; Doggett, 1988). The superior drought tolerance in the crop is likely due to its evolution in Sub-Saharan Africa, a region charecterized by predictably low and erratic rainfall patterns. Thus, it is one of the most drought tolerant crops and serves as a model for studying the genetic and physiological mechanisms of drought tolerance in cereal crop species.

Drought is consistently the most limiting abiotic stress factor to crop productivity around the world (Boyer, 1982). Developing crops to withstand the moisture stress is one the most efficient way to overcome this problem, but breeding for drought tolerance

is an extremely complex trait. Progress in developing drought tolerant germplasm through plant breeding is limited by an incomplete understanding of the genetic and physiological mechanisms that condition its expression. This effort is further hindered by highly variable environments which make evaluation inconsistent at best and contradictory at worst.

In general, potential drought tolerance mechanisms in plants include escape, avoidance and dessication tolerance (Blum, 1988). Drought response in sorghum has been classified into two distinct stages, pre-flowering and post-flowering (Rosenow, 1987). Pre-flowering drought stress leads to leaf rolling and erectness, delayed flowering and reduced height. Post-flowering drought stress causes premature leaf senescence leading to stalk lodging, stalk rot disease and significant yield loss in sorghum (Rosenow and Clark, 1995).

Understanding the genetics and physiology of the specific physioogical mechanism i.e. transpiration efficiency (change in CO_2 assimilation rate (A) per unit change in transpiration rate (E)) and its related agronomic traits that condition the photosynthetic ability of the plants under moistiure stress using the molecular markers and quantitative trait loci (QTL) analysis has the potential to develop drought tolerant sorghum.

Genetic variation for transpiration efficiency in sorghum under water limited conditions has been well documented in sorghum in both field and greenhouse studies (Krieg and Hutmacher, 1986; Kidambi et al., 1990a, Peng et al., 1991; Peng and Krieg, 1992; Krieg et al., 1992; Balota et al., 2008). However, many of these reports have

recommended further investigation of the relationships among CO_2 assimilation rate, transpiration ratio, and water use efficiency (WUE) to better elucidate the relative genetic and environmental control of the traits and the best approach to utilize this information in an improvement program.

With the advancement of molecular genotyping and QTL mapping techniques, it is now possible to dissect the multiple genetic factors that contribute to a complex trait like drought tolerance (Paterson et al., 1988). In sorghum, genetic linkage maps have been developed using genetic markers such as restriction fragment length polymorphism (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphism (AFLPs), and simple sequence repeat (SSRs), and QTLs have been detected for many agronomically important traits. Several studies have reported QTLs for plant height, maturity, yield and its component traits (Pereira and Lee, 1995; Tuinstra et al., 1998; Rami et al., 1998; Hart et al., 2001; Brown et al., 2006; Murray et al., 2008). QTLs associated with pre- and post flowering drought stress tolerance have also been identified in sorghum (Tunistra et al., 1997; Crasta et al., 1999; Subudhi et al., 2000; Xu et al., 2000; Haussmann et al., 2001; Harris et al., 2007). There have been no previous reports that associate transpiration efficiency traits to pre-flower drought tolerance with either flag leaf size and shape, leaf area, stomatal density or chlorophyll content, all believed to contribute to improvement in biomass and grain yield.

In cereal crops, morphological traits of the flag leaf and 2nd top leaf such as area, length, and width, and physiological traits such as chlorophyll content and photosynthetic capacity, are important determinants of grain yield (Chen et al., 1995;

Hirota et al., 1990). It was reported in peanut and pearl millet that specific leaf area was negatively correlated with transpiration efficiency and biomass production (Wright et al., 1994; Brown and Byrd, 1997). This is likely due to the fact that plants with low specific leaf area (thicker leaves) will have more mesophyll cells leading to higher rates of CO_2 assimilation and consequently, higher biomass production (Nelson, 1988).

With the expected global/environmental reductions in available irrigation water and increased level of atmospheric carbon dioxide, stomatal traits will play an even more important role in gas exchange in the leaf. Ultimately, the amount of CO_2 assimilated in the plants determine the amount of water lost (Raven, 2002; Poulson et al., 2006). Chen et al. (1995) demonstrated the close relationship of varietal differences in chlorophyll content to photosynthetic rates in rice. Chen et al. (1990) also reported that leaf stomatal resistance to gas diffusion influences photosynthetic and transpiration rate which are important physiological traits affecting photosynthesis. Combined, whole plant water use efficiency can be improved by enhancing leaf photosynthetic ability and its related related physiological traits.

Although the contribution of stay green to maintain high and stable yield production under post-flowering drought tolerance conditons has been well established, the genetic correlation between pre-flowering drought tolerance and agronomic traits has not been reported in sorghum. The objectives of this study were to identify co-localization of QTLs for morphological and physiological traits such as leaf area, chlorophyll content, stomatal density, biomass, grain yield and determine the relationship between these traits and transpiration efficiency in sorghum. The ultimate

goal of the study was to understand the genetic and physiological determinants of water use by relating agronomic traits to transpiration efficiency.

4.2 Material and Methods

4.2.1 Field Experiment at College Station, TX, 2009 (CS-09)

A total of 70 recombinant inbred sorghum lines and two parents (Tx430 and Tx7078) were planted in the field at College Station on 3 April 2009 and at Halfway, TX, on 28 May 2009 with 2 replications. Each genotype was planted in 6.7 m long and 0.76 m wide rows. It was intended to have plants spaced every 5 to 7 cm with the total plant population of 219,000 plant per hectare. Plant stands in our College Station field during 2009 were not uniform and had very low plant density which created large environmental effect in terms of variation in light interception, water and nutrients resulting in more tillering than we usually observe in normal planting density.

At the College Station field, measurements were collected on the following agronomic traits: plant height, days to anthesis, fresh and dry weight of stem, leaf and tillers, leaf area (LA) (LI-COR Corp., model 3100, Lincoln, NE) and SPAD readings on flag leaf (Minolta SPAD 502® chlorophyll meter, Spectrum Technologies, Inc., Illinois). Plant height was measured from ground to the tip of the panicle on the main stem and days to mid anthesis were recorded as number of days from planting until 50% of the panicle were at mid-anthesis. Separate measurements of fresh weight of leaves and stem for the main plant and the tillers were recorded by separating the leaves and stem after the harvest. Samples of each were dried at 60^{o}C for four days to record the dry weights. Leaf area (LA) was measured separately on of each the main stem leaves, the flag leaf,

and tillers using the leaf area meter. SPAD readings were made on the flag leaf of three destructively sampled plants using the SPAD meter. These measurements were made on each genotype. SPAD readings were taken to determine whether chlorophyll per unit area and total leaf chlorophyll per plant differed among genotype, and the relationship between chlorophyll, which absorbs photosynthetically active radiation, and A. These measurements were taken on three plants per genotype.

Leaf imprints were collected by smearing nail polish on 1 cm^2 of individual leaves and allowing them to dry for 15 to 20 minutes. Then white transparent tape was used to remove the dried polish from the leaf. These imprints were used to calculate stomatal density of each genotype on adaxial and abaxial regions of the leaf.

At harvest, grain yield and biomass were obtained from each genotype by randomly sampling three plants.

4.2.2 Field Experiment at Halfway, TX, 2009 (HW-09)

In Halfway, TX, measurements were made on grain yield and yield attributes. Grain yield was estimated by harvesting panicles from two meter length from each plot and separating the grain by hand threshing. It was reported by converting to g m^{-1} adjusting weight to 13% moisture level. In addition, plant height was measured from ground to tip of the main stem panicle and panicle exertion was measured from base of the flag leaf blade to the base of the main panicle. Plant stand, uniformity and desirability in terms of overall genotype performance were measured based on rating scale from 1 – 5, 1 – 4 and 1 – 9, respectively. The rating scale of '1' represent the best and higher scale represents the lower plant stand, non uniformity and hence lower desirability.

4.2.3 Greenhouse Study at College Station, TX, 2010 (GH-10)

Given the environmental variation associated with field trials, one set of the seventy RILs and the two parental lines were planted in the green house (Biotechnology Center for Crop Improvement, College Station, TX) on 18, May 2010 to produce a sample grown in a more controlled environment. Around 15 seeds per genotype were planted in a pot. After emergence, each pot was thinned to three plants. During the growth phase, pots were re-randomized at 32 and 52 days after planting. Measurements were made on the following agronomic traits; flowering time, leaf area on flag leaf, total leaf area per plant, tiller leaf area, measurements of top three leaves (length, width and area), total plant biomass (leaves and stem fresh and dry weights), and SPAD of the flag leaf and one leaf below the flag leaf during anthesis on two plants per genotype. Leaf area was measured separately on of each top four leaves on the main stem, remaining leaves and tillers leaves per plant using the leaf area meter (LI-COR Corp., model 3100, Lincoln, NE). Length and width of the leaves were obtained from leaf area meter as well. SPAD readings were made on the flag leaf and 2nd top leaf on each plant using the SPAD meter (Minolta SPAD 502® chlorophyll meter, Spectrum Technologies, Inc., Illinois) at anthesis. Leaves and stem were separated after the harvest on each plant to record the fresh weight. Dry weights were obtained by drying the plant samples in oven at 60^{o}C for three days. At grain maturity, again the measurements were made on fresh and dry weights of leaves, stem and panicle on single plant per genotype.

4.2.4 Data Analysis

Data were analyzed using the PROC GLM model in Statistical Analysis System software (SAS, version 9.1.3). The Procedure for general linear model was used to test the difference between RILs in each environment, assuming a random statistical model. The structure and evaluation of the data analysis system did not allow for combined analysis from CS-09 and GH-10 experiments. Due to the differences in traits measured in each environment, it was not possible to complete a combined analysis. The genetic map was constructed and QTL analysis was carried out using the procedure described in Chapter III.

4.3 Results

4.3.1 Phenotypic Trait Analysis

4.3.1.1 College Station, 2009 (CS-09)

The two parental lines differed with respect to all the traits measured (Table 4.1). In days to anthesis, Tx430 flowered 4 days later than Tx7078. Mean flowering time for RILs was 74 days, with a range of 67-82 days. Tx430 produced greater leaf and stem biomass on main plant as well as tillers compared to Tx7078 (Table 4.1). RILs were significantly different among each other for stem dry weight and tiller stem dry weight. Tx430 was 13 cm taller than Tx7078 and mean plant height for RILs was slightly higher than the parental lines with wide range of 64 – 173 cm (Table 4.1). Leaf color intensity on flag leafs as estimated by SPAD readings was greater for Tx430 compared to Tx7078. Mean SPAD readings on RILs was found in between the two parents. Within the RILs a wide

range of variation was observed for all the traits measured indicating the occurrence of transgressive segregation (Table 4.1).

Mean tiller leaf area on a per plant basis within the RILs was 2115.72 $cm^2 plant^{-1}$ which was slightly less than primary culm leaf area per plant (2360.69 cm^2 $plant^{-1}$). However, plant density in College Station 2009 was low and inconsistent which resulted in more tillering than normal.

Higher numbers of stomata were observed on abaxil region compared to adaxil region of the flag and 2nd top leaf (Table 4.1). Among the parents, Tx430 had more stomata on both flag and 2nd top leaf on both adaxil and abaxil region except on the adaxil region of the 2nd top leaf compared to Tx7078. Significant differences were detected among RILs for number of stomata on abaxil region of flag leaf and on both the regions of 2nd top leaf. Mean stomatal number among the RILs were 119 and 179 on the flag leaf and 129 and 197 on the second leaf on adaxil and abaxil region of the leaf, respectively.

At grain maturity, biomass produced per plant by primary culm was slightly lower compared to tillers (there were multiple tillers per plant but only one primary culm) among the RIL population (Table 4.1). However, wider ranges among the RILs for biomass and grain weight were observed indicating the greater variation for all measured traits.

Table 4.1 Trait values for Tx430 and Tx7078 parental lines and their recombinant inbred lines (RILs) at College Station, TX, during 2009.

Trait		Tx430	Tx7078	RILs mean	RILs range
Anthesis	Plant height, cm	88.2(8.6)†	75.0(0.5)	89.6 (18.2)	64.2 - 173.6
	Days to mid anthesis	79.0(1.8)	74.9(1.0)	74.2 (3.07)	67.5 - 82
	Main plant stem dry weight, g plant^{-1}	109.0(8.6)	90.9(11.4)	68.0 (16.43)	39.1 - 109.8
	Main plant leaf dry weight, g plant^{-1}	23.8(2.9)	14.8(3.8)	16.7 (5.87)	9.8 - 53.9
	Tiller stem dry weight, g plant^{-1}	70.6(17.9)	48.3(12.9)	47.4 (23.96)	8.2 - 163.4
	Tiller leaf dry weight, g plant^{-1}	28.9(14.7)	9.6(5.0)	14.0 (6.40)	3.0 - 32.0
	SPAD Flag leaf	59.5(0.7)	49.91(4.2)	56.7 (5.57)	37.8 - 70.9
	Flag leaf area, cm^2	-	-	118.7 (25.12)	72.8 - 185.5
	Main plant leaf area, cm^2 plant^{-1}	-	-	2360.6 (454.9)	1007 - 3287.9
	Tiller leaf area, cm^2 plant^{-1}	-	-	2115.7 (1042)	409.4 - 5646.3
	Exertion, cm	-	-	2.3 (3.5)	0 - 12.9
Stomatal density at anthesis					
	Flag leaf_adaxil, mm^{-2}	129.8	129.8	119.6 (24.1)	64.9 - 168.8
	Flag leaf_abaxil, mm^{-2}	181.8	168.8	179.9 (32.1)	116.8 - 266.2
	IInd top leaf adaxil, mm^{-2}	103.9	129.8	129.6 (23.3)	77.9 - 168.8
	IInd top leaf abaxil, mm^{-2}	207.7	194.8	197.7 (36.7)	123.3 - 285.7
Grain maturity					
	Panicle fresh weight, g plant^{-1}	-	-	64.2 (24.0)	22.7 - 133.8
	Tiller panicle fresh weight, g plant^{-1}	-	-	110.1 (57.8)	29.5 - 278.9
	Main plant stem fresh weight, g plant^{-1}	-	-	293.5 (90.8)	161.0 - 723.5
	Tiller stem fresh weight, g plant^{-1}	-	-	490.6 (276.5)	136.1- 1381.1
	Panicle dry weight, g plant^{-1}	-	-	58.6 (20.9)	17.8 - 115.1
	Tiller panicle dry weight, g plant^{-1}	-	-	89.1 (47.6)	19.8 - 211.4
	Stem dry weight, g plant^{-1}	-	-	90.0 (38.7)	45.1 - 307.7
	Tiller stem dry weight, g plant^{-1}	-	-	140.7 (71.5)	32.5 - 328.9
	Main plant grain weight, g plant^{-1}	-	-	36.2 (16.5)	7.2 - 75.4
	Tiller grain weight, g plant^{-1}	-	-	49.3 (33.1)	3.5 - 141.0

†Standard deviation in parenthesis

4.3.1.2 Halfway, TX, 2009 (HW-09)

Plant height measurements revealed that parent Tx430 was 8 cm taller compared to Tx7078 and with mean plant height of RILs was in between two parents and ranged from 73.6 -160 cm (Table 4.2). Tx7078 produced higher grain weight compared to Tx430. RILs showed highly significant difference among each other for grain yield with mean value of 368 g m^{-1}. Panicle exertion was greater in Tx7078 by 10 cm compared to Tx430. Mean RIL panicle exertion was 20 cm with the range of 0 – 52 cm (Table 4.2). Tx7078 had better plant stand, greater uniformity and over all greater desirability ratings compared to Tx430.

Table 4.2 Trait values for Tx430 and Tx7078 parental lines and their recombinant inbred lines (RILs) at Halfway, TX, during 2009.

Trait	Tx430	Tx7078	RILs mean	RILs range
Plant height, cm	119(14.0)†	111.5(9.1)	114.4 (19)	73.6 - 160.0
Exertion, cm	2(0.3)	5.9(2.6)	7.6 (5)	0 – 20.3
Desirability (1-9 scale)	5(0.3)	3.6(0.2)	4.6 (1)	2.5 - 9
Plant stand (1-5 scale)	2(0.7)	1.3(0.3)	1.8 (1)	1.0 - 5
Uniformity (1-4 scale)	2(0.4)	1.2(0.1)	1.6 (1)	1.0 - 4
Grain weight, g m^{-1}	119(22)	613(52)	368 (157)	72 - 785

†Standard deviation in parenthesis

4.3.1.3 Greenhouse Study, 2010

The two parental lines varied greatly with respect to leaf area, leaf length & width and fresh and dry biomass at anthesis as well as at grain maturity (Tables 4.3 and 4.4). Tx7078 produced greater leaf area on the top three leaves and total leaf area as a whole plant compared to Tx430 and the RIL population mean for leaf area was between the two parents (Table 4.3). An even wider range within the RIL population was observed for leaf area measurements at anthesis. The top three leaves were lengthier and wider in Tx7078 than Tx430 parental lines. Tx430 flowered 14 days later than Tx7078 and mean days to anthesis for the RIL population was 70 with range of 53 – 89 days. Leaf greenness estimated by SPAD readings on flag leaf and second top leaf were greater in Tx430 compared to Tx7078 (Table 4.3). With regard to fresh and dry biomass, Tx430 yielded more fresh and dry biomass than Tx7078 which was a consistent trend across all environments. However, Tx7078 produced higher tiller leaf area and tiller leaf, stem and panicle biomass compared to tillers produced by Tx430 (Table 4.4). Among the RILs, a wide range of variation was observed for each trait measured indicating sufficient variation for the traits that were measured.

Table 4.3 Trait values for Tx430 and Tx7078 parental lines and their recombinant inbred lines (RILs) at anthesis from greenhouse study at College Station, Texas during 2010.

Trait		Tx430	Tx7078	RILs mean	RILs range
Leaf Area (cm^2 $plant^{-1}$)	Flag leaf	27.4(12.1) †	74.6(26.9)	54.8 (27.6)	6.3-138.4
	2^{nd} top leaf	102.9(22.4)	160.3(18.6)	128.7 (54.5)	31.9-280.8
	3^{rd} top leaf	138.6(4.0)	167.9(9.2)	170.2 (71.9)	18.8-325.2
	4^{th} top leaf	174.7(7.7)	162.4(6.0)	194.8 (62.4)	85.4-404.3
	Total LA/plant	1156.2(526)	1301.0(509)	1301.9 (343.2)	467.5-2163.1
	Tiller LA	0	270.7	121.0 (152.1)	14.7-803.9
Leaf length (cm)	Flag leaf	20.8(6.8)	43.8(5.1)	32.3 (11.1)	11.1-61.6
	2^{nd} top leaf	40.6(4.9)	68.0(2.3)	50.3 (15.0)	19.5-92.2
	3^{rd} top leaf	53.1(2.0)	67.5(1.5)	57.7 (19.0)	13.7-116.2
	4^{th} top leaf	63.1(3.2)	62.8(0.2)	65.2 (14.1)	35.8-140.4
Leaf max width (cm)	Flag leaf	3.5(0.6)	6.9(1.1)	5.7 (1.4)	2.7-9.8
	2^{nd} top leaf	4.5(0.6)	7.0(0.3)	6.7 (1.5	3.2-10.0
	3^{rd} top leaf	5.7(0.5)	8.3(0.7)	7.6 (1.4)	4.7-11.6
	4^{th} top leaf	9.0(1.1)	7.6(2.1)	7.8 (1.3)	4.5-10.8
Flowering time (days)		80	66	70.5 (9.8)	53- 89
SPAD readings	Flag leaf	51.8(0.7)	44.1(1.1)	45.2 (4.8)	34.0 - 54.9
	2^{nd} top leaf	51.7(1.1)	46.0(1.8)	45.7 (5.0)	31.7 - 57.9
Fresh weight (g)	Leaf	42.2(1.5)	22.9(0.2)	34.5 (10.1)	15.5-53.8
	Stem	55.3(10.8)	58.4(4.9)	77.3 (24.7)	21-135.2
	panicle	9.7(6.3)	2.3(0.3)	6.9 (6.1)	1.0 - 42.9
	Total/Plant	150.0(2.5)	85(6.6)	117.9 (38.3)	31.6 - 183.7
	Tiller leaf $plant^{-1}$	0	5.6	2.5 (3.0)	0.2 - 15.7
	Tiller stem $plant^{-1}$	0	11.9	10.0 (10.4)	1.48 - 38.1
Dry weight (g)	Leaf	13.6(0.6)	7.2(0.3)	10.1 (2.7)	5.0 - 15.6
	Stem	25.3(0.1)	13.8(1.0)	19.6 (6.1)	9.0 - 38.5
	Panicle	5.3(0.1)	1.7(0.5)	3.9 (2.4)	0.8 - 13.0
	Total $plant^{-1}$	44.3(0.5)	22.8(1.2)	32.6 (9.8)	10.0 - 52.1
	Tiller leaf $plant^{-1}$	0	1.6	0.7 (0.9)	0.1 - 4.28
	Tiller stem $plant^{-1}$	0	2.3	2.5 (2.5)	0.51 - 9.6

†Standard deviation in parenthesis

Table 4.4 Trait values for Tx430 and Tx7078 parental lines and their recombinant inbred lines (RILs) at harvest from greenhouse study at College Station, Texas during 2010.

Trait		Tx430	Tx7078	RILs mean	RILs range
Fresh weight (g)					
Main plant	Leaf	43.13	20.33	22.6 (11.2) †	9.3 - 78.6
	Stem	79.74	35.8	55.3 (29.9)	18.2 - 175.8
	Panicle	8.36	3.42	5.0 (4.0)	0.7 - 25.4
	Total/plant	131.23	59.55	80.4 (43.2)	28.4 - 254
Tiller	Leaf	9.84	10.08	18.6 (10.8)	1.4 - 49.2
	Stem	24.07	49.12	37.1 (18.4)	6.5 - 89.7
	Panicle	2.82	7.04	6.3 (4.1)	0.9 - 25.7
	Total/plant	36.73	66.24	58.8 (32.0)	8.9 - 139
Dry Weight (g)					
Main plant	Leaf	18.55	8.62	11.06 (3.5)	6.3 - 26.1
	Stem	23.73	9.8	16.1 (9.0)	1.2 - 51.8
	Panicle	5.53	1.53	3.8 (3.2)	0.7 - 19.6
	Total/plant	47.81	19.95	29.8 (14.5)	8.0 - 78
Tiller	Leaf	3.01	2.6	5.9 (3.5)	0.2 - 16.4
	Stem	5.8	13.5	10.6 (5.3)	2.3 - 26
	Panicle	0.91	4.3	3.0 (2.4)	0.5- 13.4
	Total/plant	9.72	20.4	17.8 (10.1)	3.0 - 26.0

†Standard deviation in parenthesis

4.3.2 Linkage Map

In-detail description of the linkage map and identification of QTLs associated with transpiration efficiency traits were described in Chapter III.

4.3.3 QTLs for Leaf Area

Four QTLs were identified for flag leaf area; two in CS-09 on SBI-01 and SBI-06 and two in GH-10 on SBI-06 and SBI-08 (Tables 4.5, 4.6). The QTLs on SBI-06 were in close proximity in the same region of the chromosome. The phenotypic variation explained by each QTL ranged from 11- 14% with LOD score ranging from 2.8 to 3.3.

Table 4.5. Composite interval mapping of QTLs influencing leaf area, SPAD, stomatal density, biomass and grain yield at anthesis and grain maturity in grain sorghum F_6 RILs from the cross of Tx430 x Tx7078 in College Station and Halfway, TX, 2009.

Trait		QTLS	SBI#	Flanking markers	1 LOD interval (cM)	QTL position (cM)	Peak LOD score	Additive effect	R^2
CS-09: At anthesis									
Leaf area									
	Flag leaf	Flag_LA_Ant09. 1	1	DGA19-DGA20	23.6	48.2	2.8	8.70	0.12
	Flag leaf	Flag_LA_Ant09. 2	6	DGA262-DGA263	3.7	75.8	2.9	8.68	0.11
	Total LA_plant	Tot_LA_Ant09. 1	5	DGA221-DGA222	16.2	54.3	3.0	-194.61	0.17
	Total LA_plant	Tot_LA_Ant09. 2	7	DGA293-DGA294	4.3	110.2	5.5	247.08	0.26
SPAD	Flag leaf	SPAD_FL_Ant09	4	DGA168-DGA169	17.9	29.1	2.6	2.04	0.13
Stomatal density									
	Flag leaf_Adaxil	SD_FL_Adaxil_Ant09	7	DGA285-DGA288	28.9	63.6	2.9	-9.29	0.14
	Flag leaf_Abaxil	SD_FL_Abaxil_Ant09. 1	2	DGA103-DGA106	2.8	102.1	4.2	17.46	0.17
	Flag leaf_Abaxil	SD_FL_Abaxil_Ant09. 2	7	DGA271-DGA272	9.6	13.5	4.5	13.62	0.17
	2nd top leaf_Adaxil	2nd_leaf_Adaxil_Ant09	7	DGA293-DGA294	7.3	112.2	3.0	10.88	0.15
	2nd top leaf_Abaxil	SD_leaf_Abaxil_Ant09	7	DGA293-DGA294	5.6	112.2	3.0	10.88	0.15
Plant height									
	HtAnt09	Height_Ant09	6	DGA234-DGA236	11.2	8.4	3.4	-8.22	0.18
Biomass									
Main plant									
	Stem_dry_weight	StemDry_Ant09. 1	1	DGA27-DGA28	4.5	96.1	3.5	10.45	0.19
	Stem_dry_weight	StemDry_Ant09. 2	4	DGA194-DGA196	6	106.5	3.2	7.19	0.16
Tiller									
	Leaf_dry_weight	TiLeafDry_Ant09. 1	2	DGA106-DGA108	8.2	105.8	4.1	3.01	0.20
	Leaf_dry_weight	TiLeafDry_Ant09. 2	7	DGA274-DGA275	6	23.4	3.0	2.65	0.16
	Leaf_dry_weight	TiLeafDry_Ant09. 3	7	DGA282-DGA283	8.3	54.5	3.2	-2.47	0.14
	Leaf_dry_weight	TiLeafDry_Ant09. 4	9	DGA323-DGA324	1.5	0	2.9	-2.51	0.14
	Leaf_dry_weight	TiLeafDry_Ant09. 5	9	DGA327-DGA330	5.5	7.7	4.5	-2.95	0.20
	Stem_dry_weight	TiStemDry_Ant09. 1	9	DGA323-DGA329	7.5	0	2.8	-11.00	0.16
	Stem_dry_weight	TiStemDry_Ant09. 2	10	DGA390-DGA392	4.7	86.4	3.0	-11.42	0.19
CS-09: At grain maturity									
Main Plant									
	Fresh_stem	FreshStem_GM09. 1	6	DGA258-DGA259	3.8	70.7	5.3	-47.03	0.24
	Fresh_stem	FreshStem_GM09. 2	7	DGA289-DGA290	16.2	97	4.1	36.99	0.16
	Fresh_Panicle	FreshPanical_GM09	8	DGA319	13.1	85.7	3.7	10.49	0.18
	Dry_Stem	DryStem_GM09	6	DGA257-DGA258	4.8	69.5	3.1	-17.47	0.18
	Dry_Panicle	DryPanical_GM09	8	DGA317-DGA319	17.1	73.7	3.4	10.15	0.22
Tiller	Fresh_Stem	TiFreshStem_GM09. 1	1	DGA32-DGA34	12.4	117.4	4.6	-195.38	0.33
	Fresh_Stem	TiFreshStem_GM09. 2	2	DGA69-DGA70	5.1	7.2	3.9	-161.59	0.22
	Fresh_Panicle	TiFreshPanicle_GM09	7	DGA293-DGA294	2.8	109.4	3.1	-23.55	0.15
	Dry_Stem	TiDryStem_GM09. 1	2	DGA68-DGA69	6.5	3.9	3.7	-37.23	0.21
	Dry_Stem	TiDryStem_GM09. 2	10	DGA364-DGA365	7.6	33.9	3.9	35.71	0.21
Grain weight per plant									
Main plant									
	Grain weight_plant	GrainWeight_CS09.1	1	DGA48-DGA49	10.2	167.9	3.7	7.45	0.17
	Grain weight_plant	GrainWeight_CS09.2	2	DGA66-DGA68	3.3	0	2.9	6.68	0.12
Halfway-09									
	Grain weight	GrainWeight_HW09.1	6	DGA249-DGA254	3.1	60.3	4.2	64.73	0.16
	Grain weight	GrainWeight_HW09.2	6	DGA259-DGA261	9.9	71.9	3.5	64.26	0.14

Table 4.6. Composite interval mapping of QTLs influencing fresh and dry biomass, leaf area, leaf length and width at anthesis in grain sorghum F_6 RILs from the cross of Tx430 x Tx7078 under greenhouse study at College Station, TX, 2010.

Trait		QTLs	SBI#	Flanking markers	1 LOD interval (cM)	QTL position (cM)	Peak LOD score	Additive effect	R^2
Biomass									
Mainplant									
	Fresh_Leaf	Fresh_Leaf_GH. 1	3	DGA134-DGA137	11	45.2	3.20	3.83	0.13
	Fresh_leaf	Fresh_Leaf_GH. 2	6	DGA246-DGA247	10.1	49	3.22	-3.65	0.12
	Fresh_stem	Fresh_stem_GH	6	DGA246-DGA247	13.4	47	5.88	-12.45	0.23
	Fresh_Panicle	Fresh_Panicle_GH.1	1	DGA26-DGA27	10	88.9	2.68	-2.30	0.13
	Fresh_Panicle	Fresh_Panicle_GH.2	1	DGA31-DGA32	6.9	112.7	2.55	2.39	0.12
	Dry_Leaf	Dry_Leaf_GH. 1	3	DGA146-DGA147	10.1	84.4	5.27	1.25	0.19
	Dry_Leaf	Dry_Leaf_GH. 2	6	DGA246-DGA247	8.6	45	4.26	-1.44	0.19
	Dry_stem	Dry_stem_GH	6	DGA246-DGA247	16.7	49	4.72	-2.73	0.18
	Dry_Panicle	Dry_Panicle_GH	1	DGA25-DGA26	9.6	85.5	3.61	-1.04	0.16
	Total fresh biomass_plant	Total fresh biomass_GH	6	DGA246-DGA247	11.8	47	4.40	-15.85	0.19
	Total dry biomass_plant	Total dry biomass_GH. 1	4	DGA175-DGA176	12.3	71.7	3.95	-3.62	0.15
	Total dry biomass_plant	Total dry biomass_GH. 2	6	DGA246-DGA247	15.8	47	2.94	-3.23	0.12
Tiller	Fresh_Leaf	TiFreshLeaf_GH. 1	9	DGA337-DGA338	26.9	48.2	3.56	1.38	0.19
	Fresh_Leaf	TiFreshLeaf_GH. 2	7	DGA281-DGA282	8.4	49.5	4.82	-4.45	0.18
	Dry_Leaf	TiDryLeaf_GH.1	3	DGA123-DGA125	5.1	28.4	3.24	-0.35	0.15
	Dry_Leaf	TiDryLeaf_GH.2	7	DGA293-DGA294	6.4	110.2	3.48	-0.38	0.16
	Dry_Stem	TiDryStem_GH.1	7	DGA281-DGA282	6.5	49.5	4.92	-1.13	0.19
Leaf area	Flag_Leaf	FlagLA_GH.1	6	DGA249-DGA254	9.9	62.3	2.86	-10.13	0.13
	Flag_Leaf	FlagLA_GH.2	8	DGA310-DGA311	9.8	58.9	3.30	-10.55	0.14
	Total_LA_Plant	TotalLA_GH.1	3	DGA146-DGA147	10.1	85.1	4.35	154.49	0.20
	Total_LA_Plant	TotalLA_GH.2	7	DGA291-DGA293	7.2	107	3.08	125.60	0.14
	Tiller_LA	TiLA_GH	5	DGA221-DGA222	13.4	56.4	3.23	-56.85	0.13
Leaf length and width									
	3rd Leaf_Width	3rd Leaf_width_GH	9	DGA330-DGA332	4.5	11.7	3.96	0.27	0.19
	3rd Leaf_Length	3rd Leaf_Length_GH.1	7	DGA281-DGA282	6.1	49.5	3.22	-7.04	0.13
	3rd Leaf_Length	3th Leaf_Length_GH.2	7	DGA293-DGA294	6.2	110.2	3.80	-8.03	0.17
	3rd Leaf_Length	3th Leaf_Length_GH.3	8	DGA304-DGA309	7.7	44.2	4.53	-10.47	0.19
	4th Leaf_width	4th Leaf_width_GH.1	9	DGA324-DGA325	3.3	1.5	4.30	0.23	0.18
	4th Leaf_width	4th Leaf_width_GH.2	9	DGA329-DGA330	4.8	9.7	3.82	0.24	0.18
	4th Leaf_length	4th Leaf_length_GH.1	7	DGA274-DGA275	21.2	21.8	2.92	-5.49	0.14
	4th Leaf_length	4th Leaf_length_GH.2	8	DGA320-DGA322	3.8	88.9	4.03	7.00	0.20

With regard to total LA per plant, four QTLs were detected with two in CS-09 on SBI-05 and SBI-07 and two in GH-10 on SBI-03 and SBI-07. The proportion of phenotypic variation explained by these QTL ranged from 14-26% with the LOD score ranging from 3.0 – 5.5. The QTL for total LA on SBI-07 were in the same region of the chromosome in both environments. Alleles from the Tx430 increased flag leaf area in CS-09. One QTL was found with regards to tiller LA in GH-10 on SBI-05 with significant LOD score of 3.23 and explained phenotypic variation of 13%.

4.3.4 QTLs for Leaf Greenness (SPAD)

One QTL (SPAD_FL_Ant09) was detected for leaf greenness on SBI-04 in CS-09 on flag leaf. This QTL explained 13% of the phenotypic variations with the LOD score of 2.6 (Figure 4.1). The allele from Tx430 increased the leaf greenness trait in the population.

4.3.5 QTLs for Stomatal Density

In total, five QTLs were identified for stomatal density in CS-09 field (Table 4.5 and Figure 4.1) study with three QTLs on flag leaf and two on 2nd top leaf in both adaxil and abaxil region of the leaf. Among the five QTLs, four were found on SBI-07 and one on SBI-02 with the LOD score ranging from 2.9 – 4.5. The phenotypic variation explained by each QTL ranged from 14 - 17%. In all QTLs, alleles from Tx430 increased stomatal density of the leaf except on one QTL (SD_FL_Adaxil_Ant09) wherein Tx7078 decreased the stomatal density in this QTL on flag leaf.

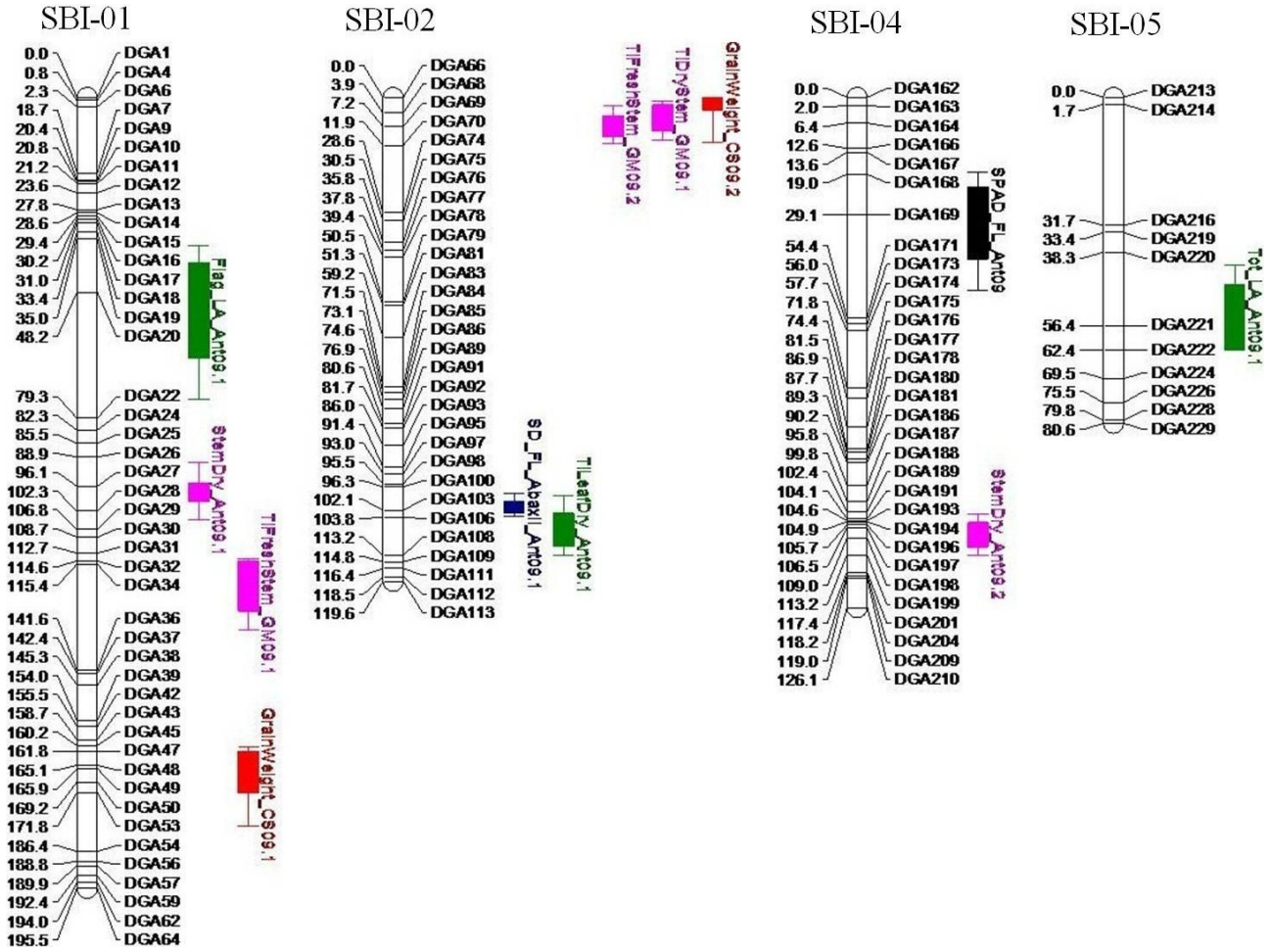

Figure 4.1.Digital Genotyping (GA-II) linkage map of sorghum showing positions of quantitative trait loci (QTLs) influencing leaf area, SPAD, stomatal density, biomass and grain weight at anthesis and grain maturity in grain sorghum.F_6 RILs from the cross of Tx430 x Tx7078 in College Station and Halfway, TX, 2009.

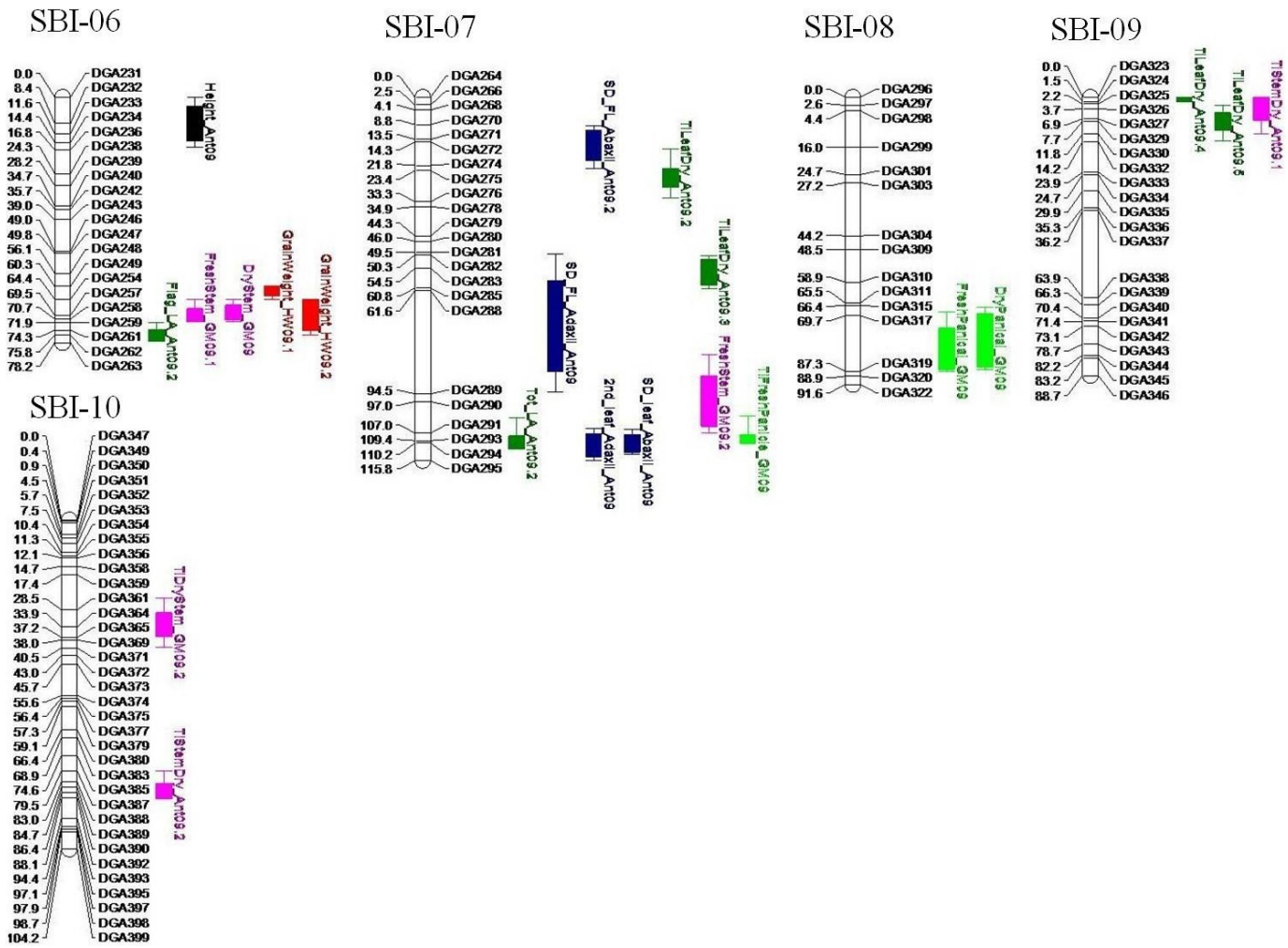

Figure 4.1. Continued

4.3.6 QTLs for Plant Height

One QTL was detected for plant height in CS-09 study on SBI-06 (Figure 4.1) in *dw3* genomic region with significant LOD score of 3.4. The phenotypic variation explained by this QTL for plant height was 18%. Alleles from Tx7078 parent contributed to shorter plant height in this population.

4.3.7 QTLs for Leaf Biomass on Main Plant

Eight significant QTLs were identified for leaf biomass on main stem in GH-10 study with three QTLs for fresh weight of leaf (two at anthesis and one at maturity) and five QTLs for dry weight of leaf (two at anthesis and three at grain maturity) (Tables 4.6, 4.7; Figure 4.1, 4. 2). Among these QTLs three were detected on SBI-06, two on SBI-07 and one each on SBI-01, SBI-03, and SBI-09 with LOD score range from 2.92 – 5.27. The phenotypic variation explained by each QTLs ranged from 9 – 19 %. Alleles from Tx430 parent increased leaf biomass in three QTLs.

4.3.8 QTLs for Stem Biomass on Main Plant

In total, ten QTLs were detected for stem biomass on main plant with five QTLs each in CS-09 (Table 4.5) and GH-10 (Tables 4.6, 4.7). Among these, four QTLs were found for fresh stem biomass (one at anthesis and three QTLs during grain maturity) and six were identified for dry stem biomass (each three during anthesis and grain maturity). Most of these QTLs (six) were found on SBI-06 and remaining four on SBI-01,SBI-04, SBI-07 and SBI-10 with significant LOD score range from 3.1 – 5.8 and the phenotypic variation explained by each QTLs ranging 16 – 24%.

Table 4.7. Composite interval mapping of QTLs influencing fresh and dry biomass at harvest in grain sorghum F_6 RILs from the cross of Tx430 x Tx7078 under greenhouse study at College Station, TX, 2010.

Trait	QTLs	SBI#	Flanking markers	1 LOD interval	QTL position	Peak LOD score	Additive effect	R^2
Main plant fresh weight								
Leaf	FreshLeafHar_GH	2	DGA103-DGA106	10.8	103.8	3.66	-4.74	0.16
Stem	FreshStemHar_GH	6	DGA246-DGA247	9.6	49.8	4.57	-14.38	0.21
Panicle	FreshPanHar_GH	2	DGA69-DGA70	26.1	11.2	2.59	-1.61	0.13
Total Biomass/palnt	TotFreshBio_Har_GH.1	2	DGA85-DGA86	5.1	74.6	3.15	-18.32	0.17
Total Biomass/palnt	TotFreshBio_Har_GH.2	2	DGA91-DGA92	3.4	81.7	4.16	-21.19	0.22
Tiller fresh weight								
Leaf	TiFreshLeafHar_GH.1	1	DGA7-DGA9	9.1	18.7	3.40	4.02	0.13
Leaf	TiFreshLeafHar_GH.2	4	DGA173-DGA174	12.5	57.7	4.42	5.04	0.18
Stem	TiFreshStemHar_GH	2	DGA66-DGA68	5.9	3.9	3.99	8.70	0.18
Panicle	TiFreshPanHar_GH.1	10	DGA351-DGA352	12.2	5.7	2.89	1.68	0.14
Panicle	TiFreshPanHar_GH.2	10	DGA372-DGA373	2.0	45.0	3.23	-2.33	0.18
Panicle	TiFreshPanHar_GH.3	10	DGA377-DGA379	7.1	59.1	2.64	1.93	0.13
Total Biomass/palnt	TiTotFreshBio_Har_GH.1	3	DGA140-DGA143	17.8	68.4	3.49	-11.99	0.15
Total Biomass/palnt	TiTotFreshBio_Har_GH.2	7	DGA291-DGA293	10.3	107.0	2.83	-11.08	0.12
Main plant dry weight								
Leaf	DryLeafHar_GH.1	1	DGA16-DGA17	3.1	31.0	4.17	-1.39	0.13
Leaf	DryLeafHar_GH.2	6	DGA246-DGA247	9.8	49.8	2.92	-1.10	0.09
Leaf	DryLeafHar_GH.3	6	DGA254-DGA257	7.0	64.4	3.16	-1.13	0.09
Stem	DryStemHar_GH.1	6	DGA248-DGA249	12.9	55.8	3.55	-4.04	0.17
Stem	DryStemHar_GH.2	10	DGA352-DGA353	7.4	7.5	3.62	4.23	0.17
Total Biomass/plant	TotDryBio_Har_GH	2	DGA91-DGA92	3.9	81.7	2.98	-5.84	0.14
Tiller dry weight								
Leaf	TiDryLeafHar_GH.1	1	DGA7-DGA9	10.9	18.7	4.06	1.57	0.18
Leaf	TiDryLeafHar_GH.2	1	DGA10-DGA11	1.2	28.6	2.78	1.37	0.13
Leaf	TiDryLeafHar_GH.3	4	DGA169-DGA171	15.6	51.1	3.83	1.80	0.22
Leaf	TiDryLeafHar_GH.4	4	DGA174-DGA175	12.0	61.7	4.93	2.07	0.27
Stem	TiDryStemHar_GH	2	DGA68-DGA69	4.7	3.9	4.08	2.59	0.19
Total Biomass/plant	TiTotDryBio_Har_GH	2	DGA68-DGA69	5.7	3.9	4.16	4.44	0.17

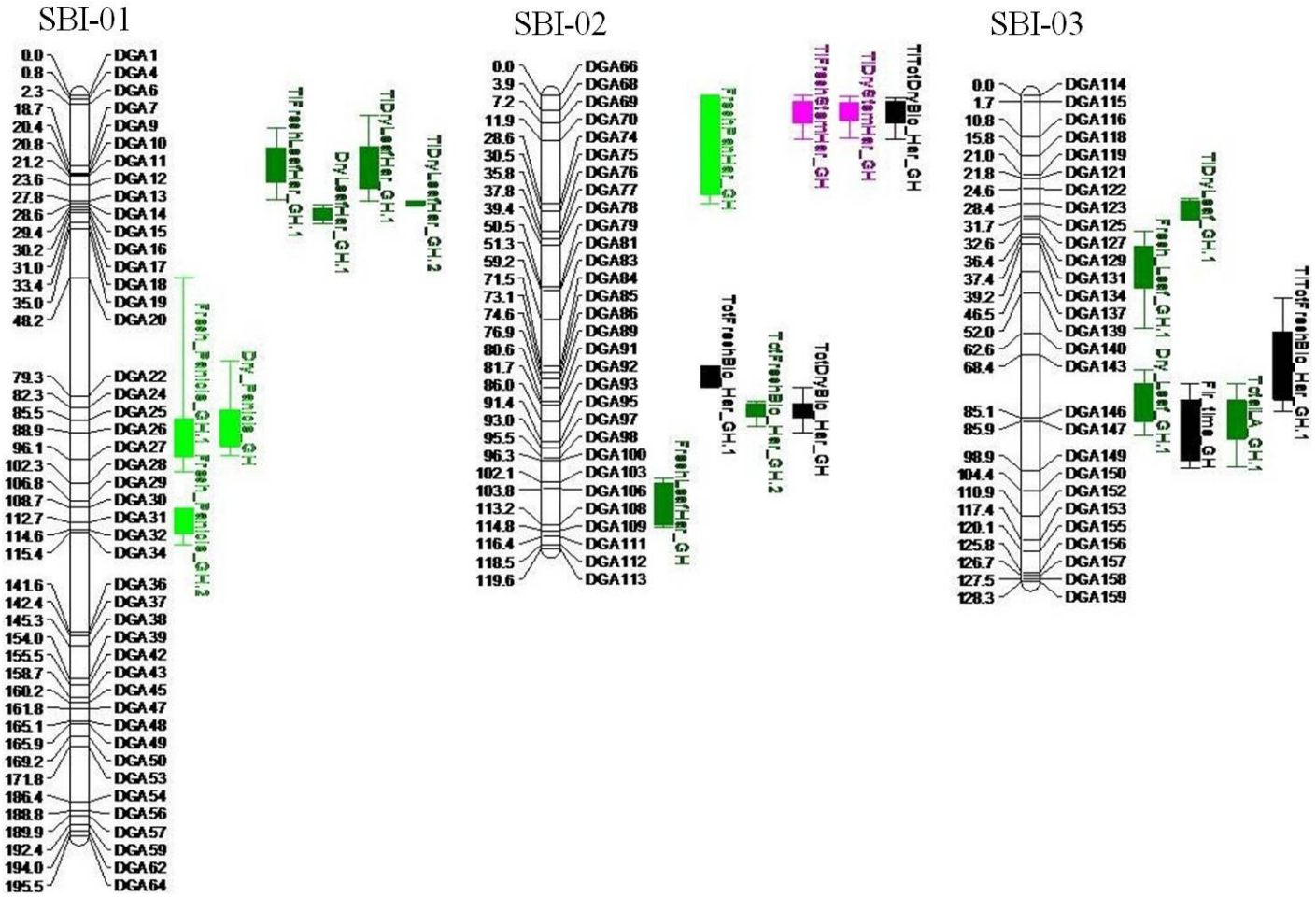

Figure 4.2.Digital Genotyping (GA-II) linkage map of sorghum showing positions of quantitative trait loci (QTLs) influencing fresh and dry biomass, leaf area, leaf length and width at anthesis in grain sorghum F_6 RILs from the cross of Tx430 x Tx7078 under greenhouse study at College Station, TX, 2010.

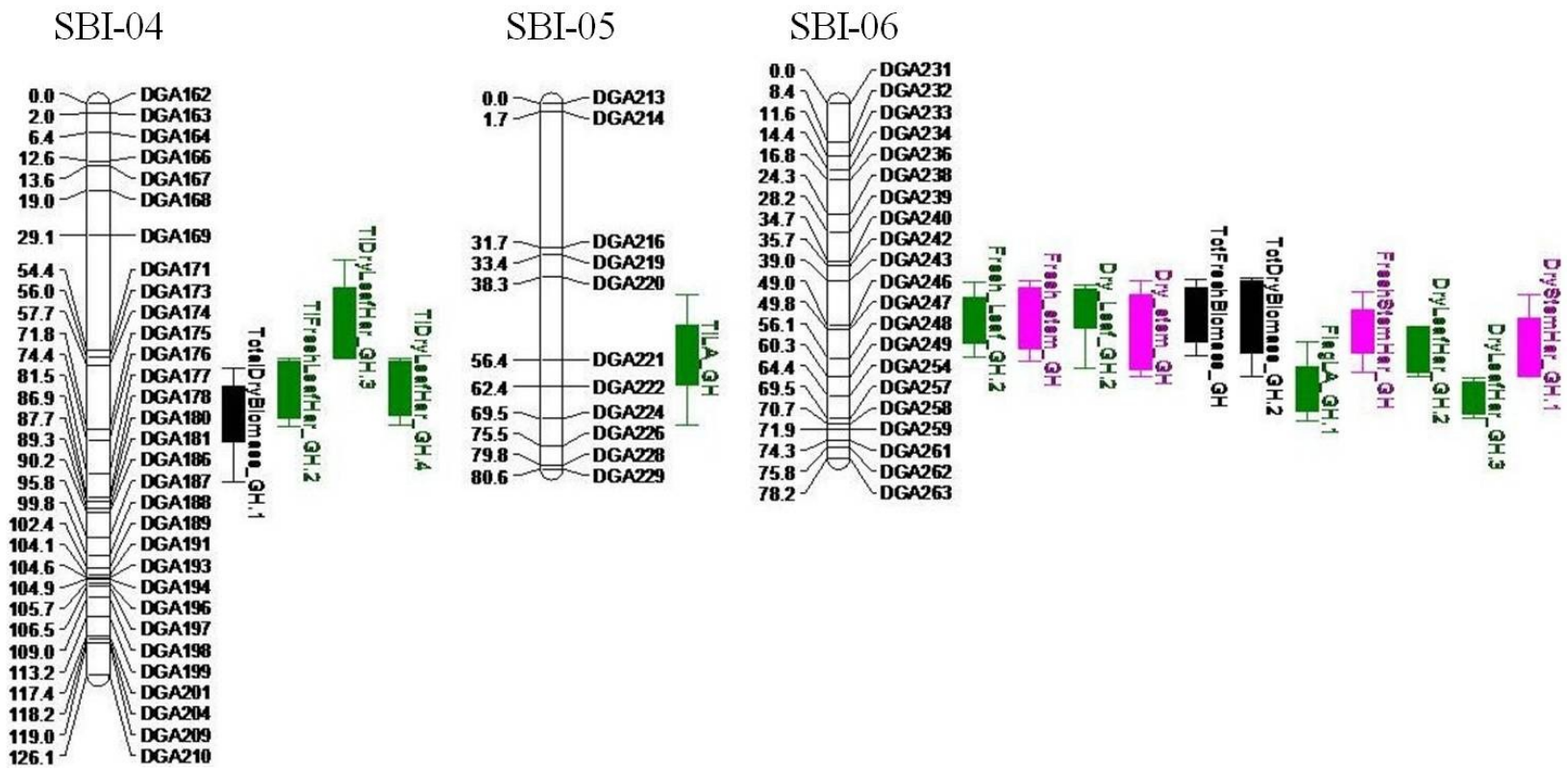

Figure 4.2. Continued

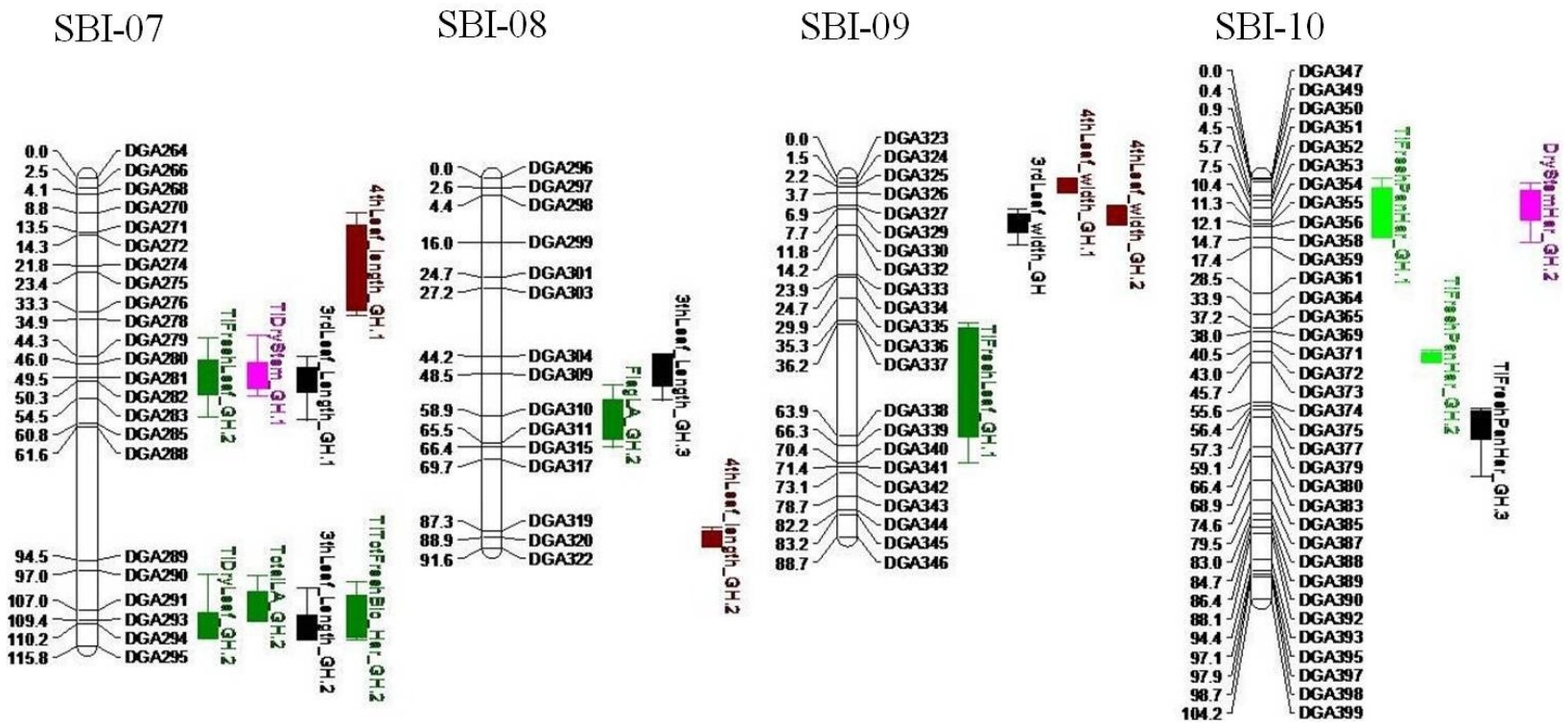

Figure 4.2. Continued

4.3.9 QTLs for Panicle Weight

Four QTLs were identified for panicle weight on main plant in GH-10 (Table 4.7 and Figure 4.2) with three QTLs during anthesis (two QTLs for fresh weight and one for dry weight of panicle) and one at grain maturity for fresh panicle weight. Among these four QTLs, three QTLs were found on SBI-01 and one on SBI-02 with LOD score ranging from 2.5 – 3.0. The phenotypic variation explained by each QTL was in the range of 12 – 17%. Alleles from the Tx7078 parent decreased panicle weight on all the QTLs found except on QTL Fresh_Panicle_GH.2.

4.3.10 QTLs for Whole Plant Biomass

Six QTLs were identified for total biomass on a per plant basis in GH-10 with each three QTLs found at anthesis and at grain maturity (Tables 4.6 & 4.7). Among these, three QTLs were detected for fresh biomass and three were related to whole plant dry biomass on a per plant basis. At harvest, all the three QTLs were found on SBI-02 and at anthesis, two QTLs were found on SBI-06 and one on SBI-04 with LOD score range of 2.9 – 4.4. The phenotypic variation explained by each QTL ranged from 12 – 24%.

4.3.11 QTLs for Leaf Length and Width

Eight QTLs were identified for leaf length and width on the 3rd and 4th top leaf in GH-10 (Table 4.6). Among these QTLs, three each were found on SBI-07 and SBI-09 and two were on SBI-08; LOD scores ranged from 2.92 – 4.53. The phenotypic variation explained by each QTL ranged from 13 – 20%.

4.3.12 QTLs for Tiller Biomass

In total 28 QTLs associated with tiller biomass characteristics were detected (Table 4.5, 4.6 and 4.7). These QTL were associated with leaf and stem fresh and dry weights and panicle weight. Twelve QTLs were detected in CS-09 (7 QTLs at anthesis and 5 at maturity) and 16 QTLs (5 QTLs detected at anthesis and 11 QTLs at grain maturity) in GH-10. These QTLs were distributed throughout the genome with the exception of SBI-04, SBI-05 and SBI-06 and LOD scores ranged from 2.78 – 4.93. The phenotypic variation explained by each QTL was ranged from 13 – 33%.

4.3.13 QTLs for Grain Weight

Four QTLs were detected for grain yield with each two QTLs in CS-09 on SBI-01 and SBI-02 based on per plant basis and HW-09 on SBI-06 based on area basis (Table 4.5). All these QTLs had LOD scores ranging from 2.9 – 4.2 and the phenotypic variation explained by each QTL ranged from 12 – 17%. Alleles from Tx430 parent increased grain yield in these QTLs.

4.4 Discussion

4.4.1 QTLs for Agronomic Traits Related to WUE

Identification of QTL controlling the important agronomic traits affecting WUE allows us to understand the genetic basis for the traits and their interaction. In this study we identified QTLs for transpiration efficiency and its related agronomic and physiological traits by using the RILs population.

Flag leaf area is an important factor which determines yield potential through affecting photosynthetic rate. Among the four QTLs identified for flag leaf area in the population, one QTL on SBI-06 was detected on the same genomic region in both environments. While this does not mean that this is the same gene but the consistency of QTL expression does make this a region of interest for further study and possibly a target for MAS.

Leaf color intensity is the important estimator of photosynthetic capacity because it determines CO_2 assimilation rate. In the present study, a single QTL was detected for leaf greenness (SPAD meter reading) on chromosome 4 in flag leaf and it explained 13% of the phenotypic variation.

Stomatal density plays an important role in adjusting with change in availability of water and atmospheric CO_2. Anderson and Briske (1990) reported that density and size of the stomata are positively related to transpiration, thus play an important role under water stress conditions. Among the five QTLs identified in this study for stomatal density on flag leaf and 2^{nd} top leaf, four were found on SBI-07, suggesting that this region of the genome is quite important for this trait.

Leaf photosynthesis and its related physiological traits are the major targets to be improved in sorghum physiological breeding to attain drought tolerant genotypes. In the present study, several significant QTLs were identified for leaf and stem biomass during anthesis as well as at maturity. The majority of these QTLs were found in a cluster on SBI-06 with leaf area and yield components, indicating that same gene may be involved in controlling these traits. In addition, significantly positive correlation was observed between the transpiration efficiency and both grain and biomass yield. However, transpiration efficiency was negatively correlated with stomatal density and leaf area.

4.4.2 QTLs for Grain Weight and Its Components

Two highly significant QTLs were identified for grain weight in both environments (CS-09 and HW-09) on SBI-01, SBI-02 and SBI-06 and they explained about 12 – 17% of phenotypic variation. QTL for grain weight on chromosome SBI-06 which explained 14% of the phenotypic variation in this study was also reported by Srinivas et al., (2009) in sorghum. In the present study, the lower population size minimizes the power to detect small effect QTL and likely increases the effect of subsampling on the observed results. There are likely many other QTLs affecting the grain weight per plant that were not detected due to smaller effects and were undetected at the significant QTL identification threshold. The QTL for grain weight on SBI-01 & SBI-02 have not been previously reported indicating that presence of additional genetic loci controlling the trait in the population. Four QTLs were identified for panicle weight on primary culm in GH-10 with three QTLs positioned on SBI-01 and one on SBI-02 and phenotypic

variation explained by each QTL was in the range of 12 – 17%. On SBI-02, both panicle weight and grain weight share the same genomic region of the chromosome.

4.4.3 Clustering of QTLs Affecting the Agronomic Traits

Clustering of QTLs for different traits is more likely due to linkage of many genes affecting the traits since genes are often located in the gene-rich regions with hot spots of recombination (Gill et al. 1996; Faris et al.2000; Sandhu et al. 2001), or due to pleiotropic effect i.e. a single gene affect multiple traits (Veldboom et al. 1994; Xiao et al. 1996).

In the present study, we observe the common phenomenon of QTL clustering. Major QTL clusters of about 5, 15, 5, 1.5 cM regions were located on SBI-02, SBI-06, SBI-07 and SBI-09, respectively (Figure 4.3). On SBI-02, eight traits such as tiller stem fresh and dry biomass, fresh panicle weight and grain weight were found significantly associated on the same region of the chromosome. At this genomic region, alleles from Tx430 contributed to improvement of grain weight. QTL cluster on the SBI-06 were found to be significantly associated with twelve agronomic traits related to biomass (leaf, stem and total biomass) and leaf area. On SBI-07 within the 5 cM region, nine traits were found on the same region of the chromosome including transpiration rate, total leaf area, stomatal density, leaf length, leaf and stem biomass. Transpiration efficiency trait was found to coincide with leaf and stem biomass, leaf length and width on SBI-09 within the 3 cM region. Therefore, these traits could be used as indirect selection for transpiration efficiency. Higher the leaf length and width, greater the

number of stomata, thereby higher CO_2 assimilation rate which results in greater transpiration efficiency.

Several studies (Srinivas et al., 2009; Graham and Lessman, 1996; Casady, 1965; Rami et al., 1998) reported the QTLs clustering for different agronomic traits in sorghum. Srinivas et al.(2009) reported the major QTL cluster of about 7 cM region on the chromosome SBI-06, near the *Glume* type gene with significant association of seven traits such as plant height, days to anthesis, green leaf area, panicle length and grain yield in RIL population obtained from the cross between 296B x ISI18551. Further, they predicted that this genomic region was conditioned by *Dw2* gene for plant height. Graham and Lessman (1996) reported the pleotropic effect of *Dw2* gene on panicle length, yield, seed weight and leaf area in two isogenic lines of sorghum differing at *Dw2* loci. Pleiotropic effects of *Dw3* gene on kernel weight, number of kernel per panicle, tiller number and panicle size was reported by Casady (1995). Rami et al. (1998) also reported major QTL cluster for germination rate, number of kernel per panicle, grain yield, seed weight, panicle compactness, plant height and panicle length at the *Dw3* chromosomal region on linkage group A (SBI-07).

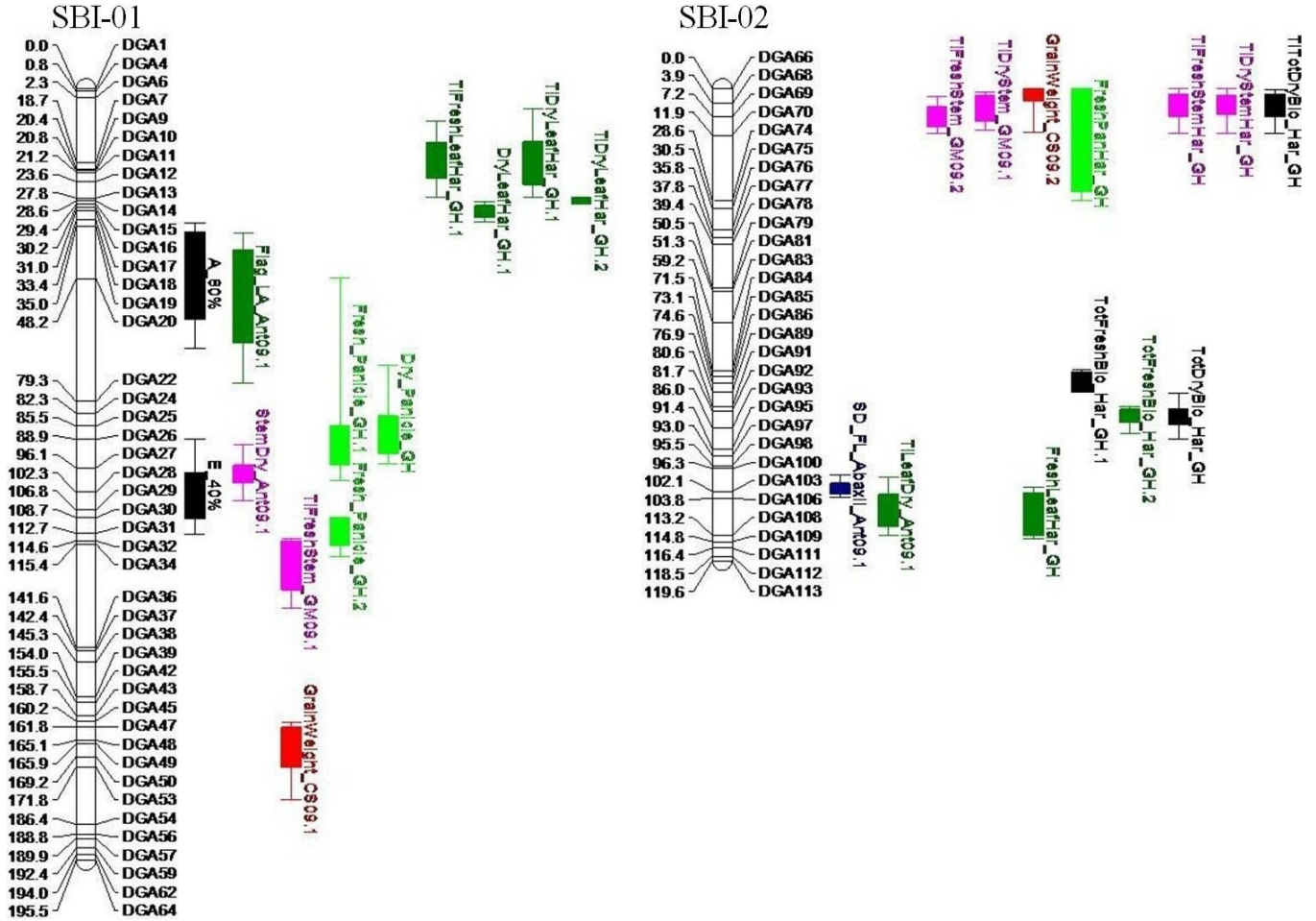

Figure 4.3. Digital Genotyping (GA-II) linkage map of sorghum showing co-localization of quantitative trait loci (QTLs) for transpiration efficiency and related agronomic traits from the combined studies of greenhouse, Bushland,TX, 2008, CS-09 and GH-10. The map was developed using the F_6 RIL population of the cross Tx430 x Tx7078.

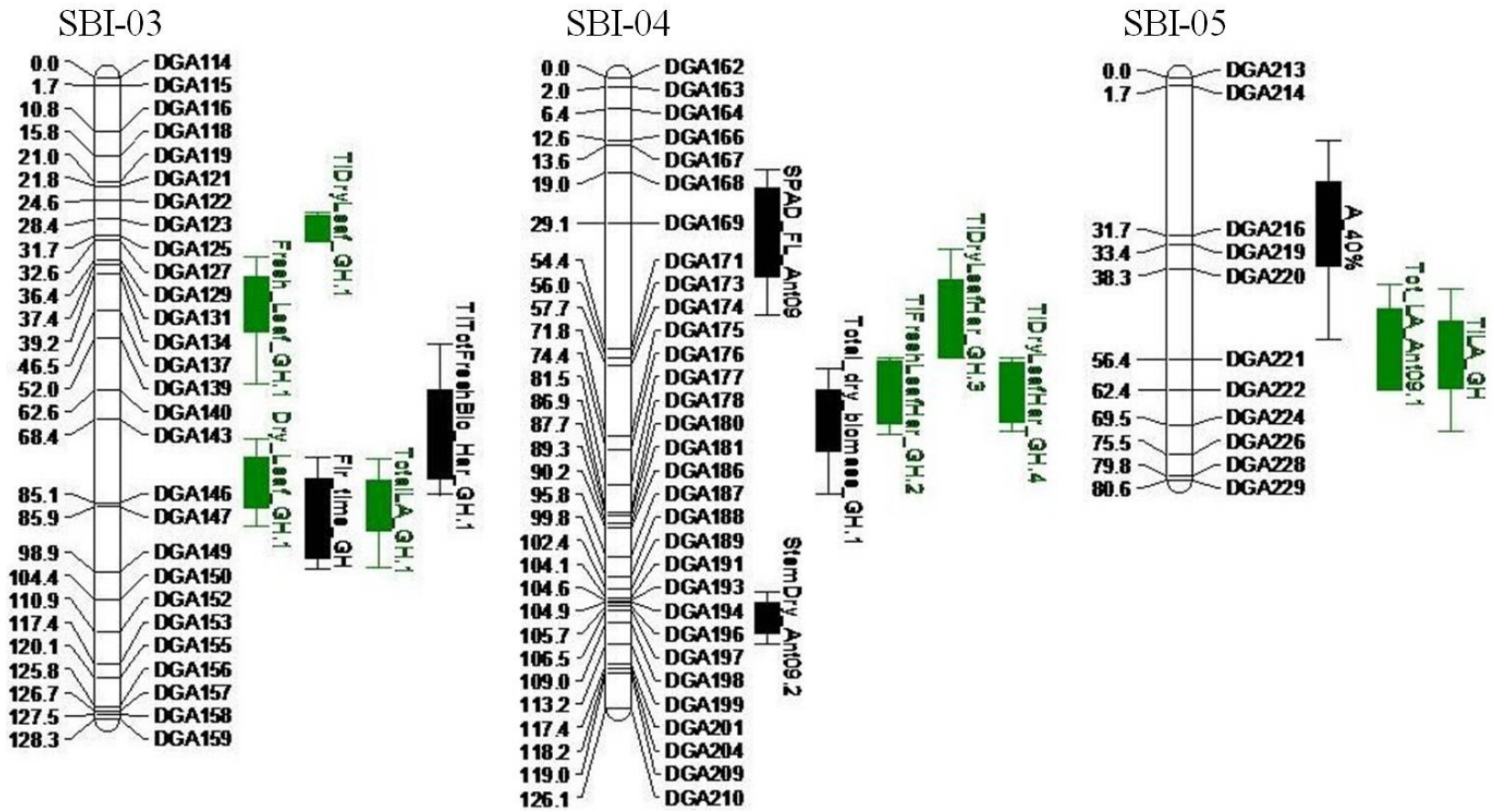

Figure 4.3. Continued

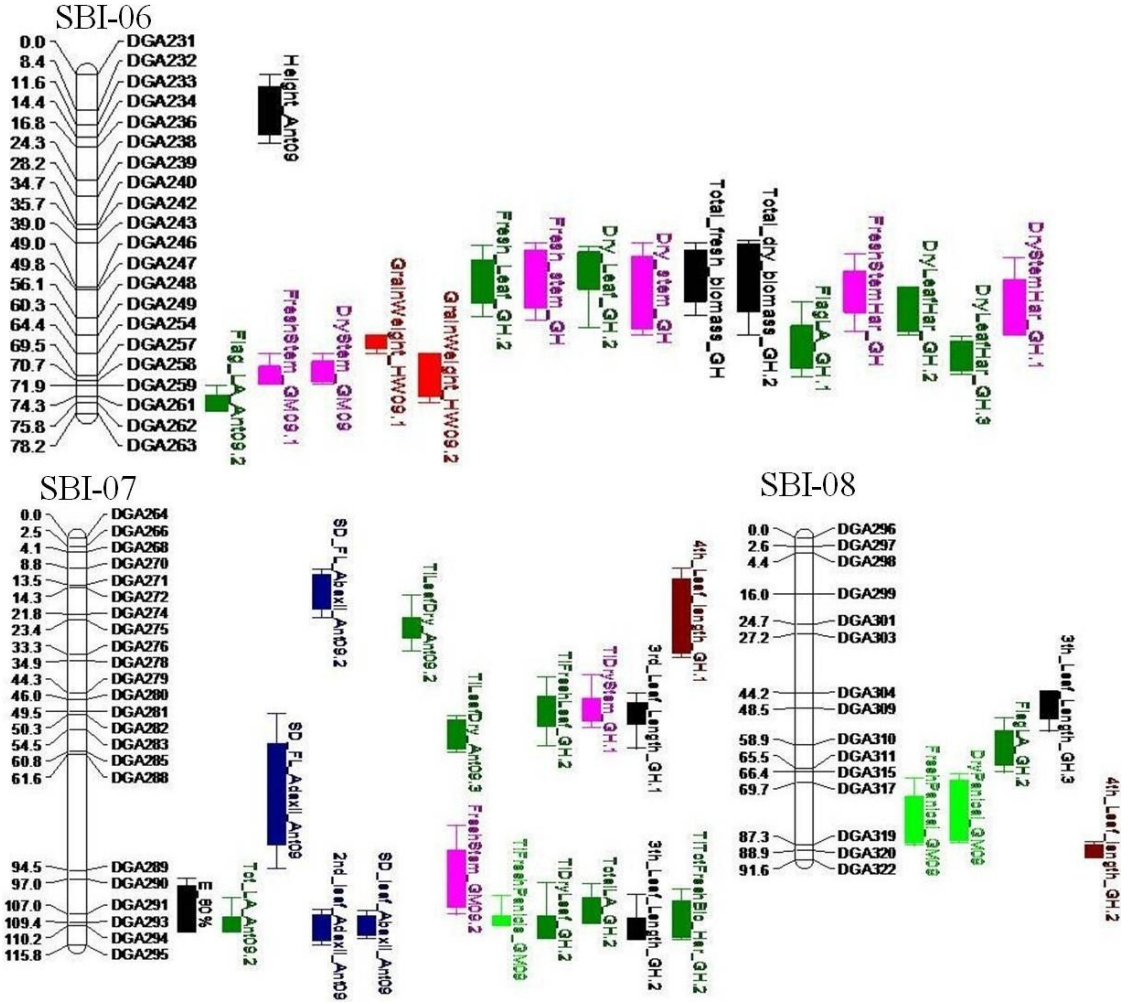

Figure 4.3. Continued

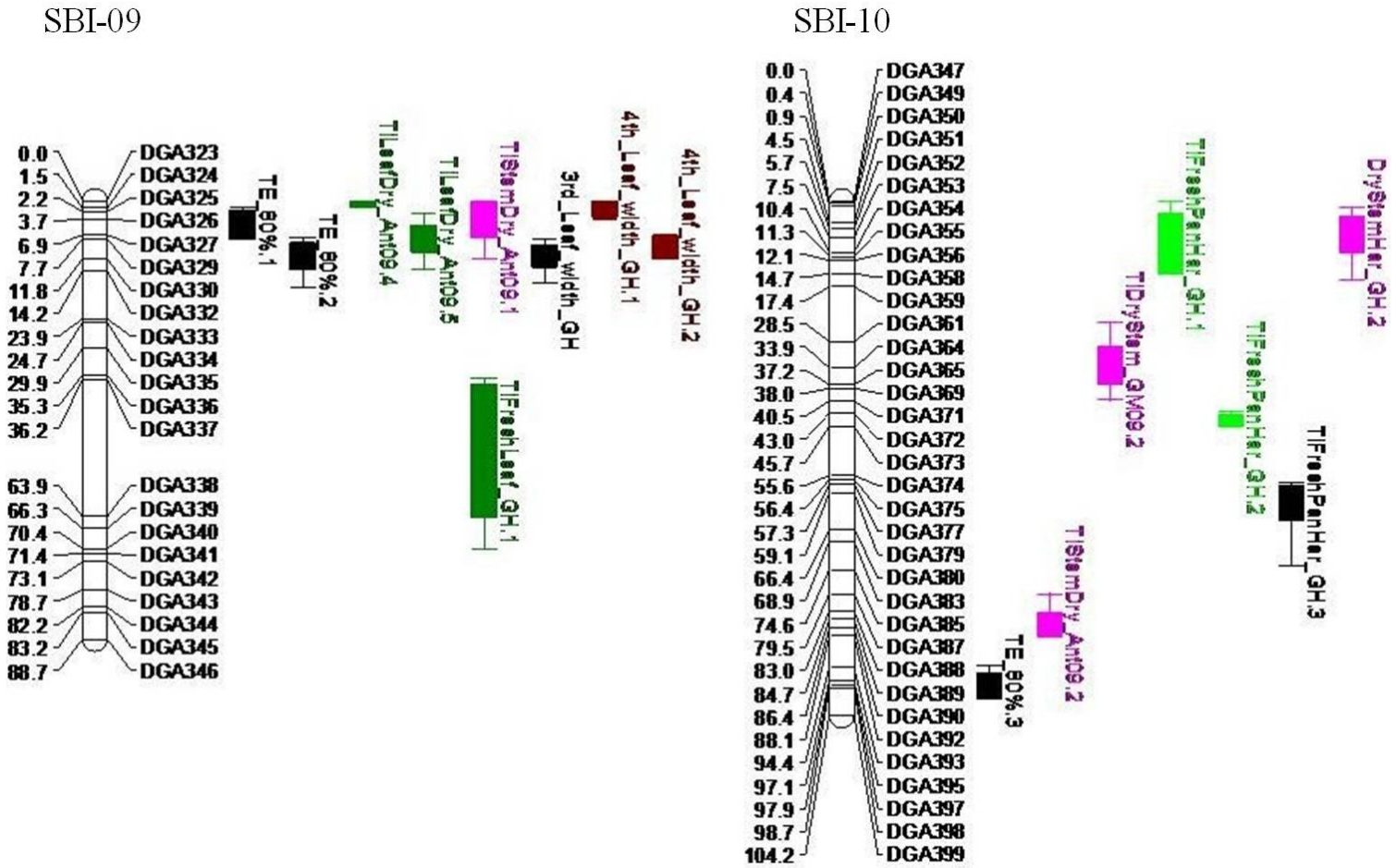

Figure 4.3. Continued

As mentioned earlier, in the current study clustering of QTLs for agronomic traits might be due to tight linkage of the genes or pleiotropy of a single gene. However, to correctly understand the genetic basis of the association between these traits, more detailed studies such as increasing the population size and saturating the target genomic regions by obtaining the high resolution genotypic data to add more markers using near-isogenic lines (NILs) for the QTLs would be needed. This might lead to map-based cloning of genes underlying the QTLs.

4.4.4 Co-localization of QTLs Associated with Transpiration Efficiency and Agronomic Traits

For transpiration efficiency, which measures the amount of biomass produced per unit water transpired through plants, three significant (LOD > 3.9) QTLs were detected on SBI-09 and SBI-10 under the 80% water regime. Two QTLs on SBI-09 accounted for 19% and 21% and QTL on SBI-10 accounted for 17% of the total phenotypic variation for the transpiration efficiency trait. Two QTLs (A_40% and A_80%) influencing CO_2 assimilation rate were identified on SBI-05 and SBI-1 which contributed 12- 15 percent of the phenotypic variability. Two QTLs (E_40% and E_80%) were also detected for transpiration rate on SBI-01 and SBI-07 under both 40% and 80% water regime which together contributed 25% of the phenotypic variability.

In the present study, multiple clusters of QTLs were identified relating to transpiration efficiency and agronomic traits where coincidence of position suggests that there may be genes in common that influence these traits. Transpiration efficiency QTLs (TE_80%.1 and TE_80%.2) co-localized with leaf and stem biomass QTLs, specifically,

3rd and 4th top leaf width QTLs were detected on SBI-09 (Figure 4.3). QTL for CO_2 assimilation rate (A_80%) coincides with the QTL position of flag leaf area (Flag_LA_Ant09.1) on SBI-01 during anthesis. On the same chromosome transpiration rate QTL (E_40%) was found to overlay with QTLs for stem dry biomass at anthesis, tiller fresh stem biomass at grain maturity, fresh and dry panicle weight. Another transpiration rate QTL (E_80%) identified on SBI-07 co-localized with QTLs for total leaf area, stomatal density on abaxil region of flag leaf and adaxil region of 2nd top leaf during anthesis. These results indicated that transpiration efficiency QTLs are at least associated with leaf area, biomass (main plant and tiller), leaf width and stomatal density. While this might be due different genes in the same region of the chromosome, it could also indicate the pleiotrophic effects of a single gene. Co-localization of these agronomic traits with transpiration efficiency indicates the strong association of agronomic traits with photosynthesis in relation to water use efficiency and biomass production. Strong genetic association of these agronomic traits with transpiration efficiency indicates the possibility of considering for indirect selection in breeding for pre-flower drought tolerance provided they have high heritability. Moreover the measurements of these agronomic traits are easy and rapid as against the LICOR measurement for transpiration efficiency.

4.5 Conclusions

This study was unique as it investigated the genetic basis of transpiration efficiency and its relationship with agronomic traits in sorghum. The majority of the QTLs regulating the traits in the study were highly significant and several QTLs were identified consistently in both field and greenhouse experiments indicating that they are highly reliable. Further, co-localization of transpiration efficiency QTLs with agronomic traits such as leaf area, biomass (main plant and tiller), leaf width and stomatal density, indicates that these agronomically important QTLs can be used for further improving the sorghum performance through marker assisted selection (MAS) strategies under pre-flowering drought stress condition.

CHAPTER V

SUMMARY

The main goal of the study was to estimate the genetic variation for transpiration efficiency (A:E) trait related to pre-flower drought tolerance in a sorghum RIL population and to identify the QTLs associated with these traits.

A greenhouse study was conducted at Bushland, TX, 2008, using the 70 RIL population derived from cross between Tx430 and Tx7078 which two sorghum inbred lines with contrasting transpiration ratios (Balota et al., 2008). The experimental design was a randomized complete block with replications, genotype and water regime (40 and 80 per cent of water regime) as experimental factors. Gas exchange traits were measured at leaf level using the LI-6400 at 31 days after planting four days and total biomass were obtained at 35 days after planting to obtain whole plant water use efficiency.

The results from the study provide the evidence that there is genetic variability among genotypes for gas exchange traits during pre-flowering drought stress. Heritability estimates based on individual environments for A:E , CO_2 assimilation rate (A) and transpiration rate (E) were 0.77, 0.45, 0.37 and 0.90, 0.33, 0.71 under 80% and 40% water regime, respectively. High correlation between the transpiration efficiency vs. CO_2 assimilation rates and whole plant WUE vs. total biomass per plant were similar to the results reported by Balota et al. (2008). Based on this information, it is logical to believe that these results are potentially useful to identify genetic loci that may be associated with pre-flower drought tolerance.

A genetic map was constructed using the digital genotyping method that collects information on polymorphic sequences from specific sites across the sorghum genome using Illumina GAII sequencer. The total length of the genetic map was 1128.6 centiMorgans (cM in the kosambi function), with total of 261 informative addition and deletion polymorphic markers were used from 3X cluster analysis. Using the composite interval mapping, we identified three highly significant QTLs associated with transpiration efficiency trait, two on SBI-09 and one on SBI-10 with one LOD interval length ranging from 5.3 to 5.7 cM and accounted for 17% - 21% of the phenotypic variation. This provides an opportunity to explore the genes which are already identified for specific physiological mechanisms within the 5.3 Mbp genomic regions. If so, we can utilize this information to enhance selection in the breeding program.

Further, these RILs and two parents were planted in the field at College Station and at Halfway, TX, during 2009 with 2 replications and one set of RILs at greenhouse, College Station, TX, during 2010 to understand the genetics and physiology of water use by relating agronomic traits to transpiration efficiency. At the College Station field, measurements were collected on the following agronomic traits: plant height, days to anthesis, fresh and dry weight of stem, leaf and tillers, leaf area, SPAD readings on flag leaf and stomatal density on adaxil and abaxil regions of flag leaf and 2^{nd} top leaf. At Halfway, data were collected on yield and some breeding traits such as plant height, exertion, plant stand, uniformity and desirability. In the greenhouse study at College Station, TX, during 2010, another set of data were collected on these agronomic traits under a more controlled environment.

The results from the study indicated that two parental lines differed with respect to all the traits measured and within the RILs wide range of variation was observed for all the traits indicating the occurrence of transgressive segregation. Numerous QTLs were identified for each individual trait within the RILs. The majority of the QTLs regulating the traits in the study were highly significant and several QTLs were identified consistently in both filed and greenhouse experiments indicating that they are highly reliable. Further, we noticed the co-localization of transpiration efficiency QTLs with agronomic traits such as leaf area, biomass (main plant and tiller), leaf width and stomatal density, indicating that these agronomically important QTLs can be used for further improving the sorghum performance through marker assisted selection (MAS) strategies under pre-flowering drought stress condition.

REFERENCES

Anderson, V. J. and D.D. Briske. 1990. Stomatal distribution, density and conductance of three perennial grasses native to the southern true prairie of Texas. The American Midland Naturalist J. 123:152-159.

Bacon, M.A. 2004. Water use efficiency in plant biology. Blackwell Publishing Ltd., Boca Raton, FL.

Balota, M., W.A. Payne, W.L. Rooney, and D.T. Rosenow. 2008. Gas exchange and transpiration ratio in sorghum. Crop Sci. 48:2361-2371.

Bandaru, V., B.A. Stewart, R.L. Baumhardt, S. Ambati, C.A. Robinson, and A. Schlegel. 2006. Growing dryland grain sorghum in clumps to reduce vegetative growth and increase yield. Agron. J. 98:1109–1120.

Bennet, W.F., B.B. Tucker, and A.B. Maunder. 1990. Modern grain sorghum production. Iowa State Univ. Press, Ames.

Bhargava, S., S. Paranjpe. 2004. Genotypic variation in the photosynthetic competence of *Sorghum bicolor* seedlings subjected to polyethylene glycol-mediated drought stress. J. Plant Physiol. 161:125–129.

Blum, A. 1979. Genetic improvement of drought resistance in crop plants: A case for sorghum. *In* H. Mussell and R.C. Staples (eds.) Stress physiology in crop plants. New York;Wiley Interscience. p.429-445.

Blum, A. 1988. Plant breeding for stress environments. CRC Press: Boca Raton, FL.

Blum, A. 2004. Sorghum physiology. p. 141–223. *In* H.T. Nguyen and A. Blum (ed.) Physiology and biotechnology integration for plant breeding. Marcel Dekker, New York.

Borrell, A. K., D. Jordan, J.E. Mullet, P.E. Klein, R. Klein, H.T. Nguyen, D.T. Rosenow, G. Hammer, A. Douglas, and B. Henzell. 2004. Discovering stay-green drought tolerance genes in sorghum: a multidisciplinary approach. *In*: T. Fischer, N. Turner, J. Angus, L. McIntyre, M. Robertson, A. Borrell and D. Lloyd, New Directions for a Diverse Planet: Proceedings of the 4th International Crop Science Congress. Brisbane, Australia, 26 September-1 October 2004. p. 1-5.

Boyer, J.S. 1982. Plant productivity and environment. Science. 218:444 – 448.

Brown, P.J., P.E. Klein, E. Bortiri, C.B. Acharya, W.L. Rooney, and S. Kresovich. 2006. Inheritance of in xorescence architecture in sorghum. Theor Appl Genet. 113:931–942.

Brown, R.H., and G.T. Byrd. 1997. Transpiration efficiency, specific leaf weight, and mineral concentration in peanut and pearl millet. Crop Sci. 37:475-480.

Casady, A.J. 1965. Effect of single height (Dw) gene of sorghum on grain yield, grain yield components and test weight. Crop Sci. 5:385–389.

Chen, W.F., Z. Xu, and L. Zhang. 1995. Physiological bases of super high yield breeding in rice. Liaoning Science and Technology Publishing Company, Shenyang, China.

Chen, W.F., Z.J. Xu, L. Zhang, and S.R. Yang. 1990. Comparative studies on stomatal density and its relations to gas diffusion resistance and net photosynthetic rate in rice leaf. Chin J. Rice Sci. 4:163-168.

Condon, A.G., R.A. Richards, G.J. Rebetzke, and G.D. Farquhar. 2004. Breeding for high water-use efficiency. J. Experimental Botany. 55:2447–2460.

Crasta, O.R, W.W. Xu, D.T. Rosenow, J.E. Mullet, and H.T. Nguyen. 1999. Mapping of post-flowering drought tolerance traits in grain sorghum: association between QTLs influencing premature senescence and maturity. Mol Gen Genet. 262:579–588.

Doggett, H. 1998. Sorghum. Second Ed. London: Longman Scientific and Technical.

Donatelli, M., G.L. Hammer, and R.L. Vanderlip. 1992. Genotype and water limitation effects on phenology, growth, and transpiration efficiency in grain sorghum. Crop Sci. 32:781–786.

Eastin, J.D., T.E. Dickinson, D.R. Krieg, and A.B. Maunder. 1983. Crop physiology in dryland agriculture. p. 334-364. *In* H.E. Dregne and W.O. Willis (ed). Dryland agriculture. 1st ed. Agric. Monogr. 23. ASA, CSSA, and SSSA, Madison, WI.

FAO (Food and Agricultural Organization of the United Nations), 2002. Crops and drops: Making the best use of water for agriculture. FAO, Rome.

FAO (Food and Agriculture Organization of the United Nations), 2009. Preliminary 2009 data on sorghum area, production and productivity. Available at http://faostat.fao.org /site/567/DesktopDefault.aspx?PageID=567#ancor. FAO, Rome.

Faris, J.D., K.M. Haen, and B.S.Gill. 2000. Saturation mapping of a gene-rich recombination hot spot region in wheat. Genetics. 154:823–835.

Gill, K.S., B.S. Gill, T.R. Endo, and T. Taylor. 1996. Identification and high density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics. 144:1883–1891.

Graham, D., and K.J. Lessman. 1966. Effect of height on yield and yield components of two isogenic lines of *Sorghum vulgare*. Crop Sci. 6:372–374.

Hamdy, A., R. Ragab, E. Scarascia-Mugnozza. 2003. Coping with water scarcity; water saving and increasing water productivity. Irrigation and Drainage. 52:3-20.

Hammer, G.L., G.D. Farquhar, and I.J. Broad. 1997. On the extent of genetic variation for transpiration efficiency in sorghum. Aust. J. Agric. Res. 48:649–655.

Harris, K., P. K. Subudhi, A. Borrell, D. Jordan, D.T. Rosenow, H.T. Nguyen, P.E. Klein, R. Klein, and J.E. Mullet. 2007. Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. J. Exp. Botany. 58(2):327–338.

Hart, G.E., K.F. Schertz, Y. Peng, and N. Syed. 2001. Genetic mapping of *Sorghum bicolor* (L.) Moench QTLs that control variation in tillering and other morphological characters. Theor Appl Genet. 103:1232–1242.

Haussmann, B.I.G., V. Mahalakshmi, B.V.S. Reddy, N. Seetharama, C.T. Hash, and H.H. Geiger. 2002. QTL mapping of stay-green in two sorghum recombinant inbred populations. Theor. Appl. Genet. 106:133–142.

Henderson, S.A., S. Von Caemmerer, G.D. Farquhar, L.J. Wade, and G.L. Hammer. 1998. Correlation between carbon isotope discrimination and transpiration efficiency in lines of the C4 species *Sorghum bicolor* in the glasshouse and the field. Aust. J. Plant Physiol. 25:111–123.

Hirota, O., M. Oka, and T. Takeda. 1990. Sink activity estimation by sink size and dry matter increase during the ripening stage of barley (*Hordeum vulgare*) and rice (*Oryza sativa*). Annals of Botany. 65(4):349-354.

Howell, T.A. 2001. Enhancing water use efficiency in irrigated agriculture. Agron. J. 93:281–289.

Iwata, H., and S. Ninomiya. 2006. AntMap: Constructing genetic linkage maps using an ant colony optimization algorithm. Breeding Science. 56:371-377.

Kassahun, B., F.R. Bidinger, C.T. Hash, and M.S. Kuruvunashetti. 2010. Stay-green expression in early generation sorghum (*Sorghum bicolor* (L.) Moench) QTL introgression lines. Euphytica. 172: 351 – 362.

Kebede, H., P.K. Subudhi, D.T. Rosenow, and H.T. Nguyen, 2001. Quantitative trait loci influencing drought tolerance in grain sorghum (*Sorghum bicolo*r L.Moench). Theor Appl Genet. 103:266-276.

Kidambi, S.P., D.R. Krieg, and D.T. Rosenow. 1990a. Genetic variation for gas exchange rates in grain sorghum. Plant Physiology. 92:1211–1214.

Kidambi, S.P., D.R. Krieg, and H.T. Nguyen. 1990b. Parental influences on gas exchange rates in grain sorghum. Photosynthetica. 50:139-146.

Kim, J.S., P.E. Klein, R.R. Klein, H.J. Price, J.E. Mullet, and D.M. Stelly. 2005. Chromosome identification and nomenclature of *Sorghum bicolor*. Genetics. 169:1169-1173.

Knapp, S.J., W.W. Stroup, and W.M. Ross. 1985. Exact confidence intervals for heritability on a progeny mean basis. Crop Sci. 25: 192 – 194.

Krieg, D.R., and H.B. Hutmacher. 1986. Photosynthetic rate control in sorghum: Stomatal and nonstomatal factors. Crop Sci. 26:112–117.

Krieg, D.R., F.S. Girma, and S. Peng. 1992. No evidence of cytoplasmic male-sterility systems influencing gas exchange rate of sorghum leaves. Crop Sci. 32:1342–1344.

Li, Z., S.R.M. Pinson, J.W. Stansel, and A.H. Paterson. 1998. Genetic dissection of the source-sink relationship affecting fecundity and yield in rice (*Oryza sativa* L.). Molecular Breeding. 5:419-426.

Lin, Y.R., K.F. Schertz, and A.H. Paterson. 1995. Comparative analysis of QTLs affecting plant height and maturity across the poaceae, in reference to an interspecific sorghum population. Genetics. 141: 391 – 411.

Menz, M.A., R.R. Klein, J.E. Mullet, J.A. Obert, N.C. Unruh, and P.E. klein. 2002. A high-density genetic map of *Sorghum bicolor* (L.) Moench based on 2926 AFLP, RFLP, and SSR markers, Plant Mol. Biol. 48:483-499.

Mortlock, M.Y., and G.L. Hammer. 1999. Genotype and water limitation effects on transpiration efficiency in sorghum. J. Crop Prod. 2:265–286.

Murray, S.C., A. Sharma, W.L. Rooney, P.E. Klein, J.E. Mullet, S.E. Mitchell, and S. Kresovich. 2008. Genetic improvement of sorghum as a bio-fuel feedstock: I. QTL for stem sugar and grain nonstructural carbohydrates. Crop Sci. 48:2165-2179.

Nelson, C.J. 1988. Genetic association between photosynthetic characteristics and yield: review of evidence. Plant Physiology and Biochemistry. 26:543–554.

Paterson, A.H., E.S. Lander, J.D. Hewitt, S. Peterson, S.E. Lincoln, and S.D. Tanksley. 1988. Resolution of quantitative traits into Mendelian factors by using a complete RFLP linkage map. Nature. 335:721–726.

Peng, S., and D.R. Krieg. 1992. Gas exchange traits and their relationship to water use efficiency of grain sorghum. Crop Sci. 32:386–391.

Peng, S., D.R. Krieg, and F.S. Girma. 1991. Leaf photosynthetic rate is correlated with biomass and grain production in grain sorghum lines. Photosynth. Res. 28:1–7.

Pereira, M.G., M. Lee, 1995. Identification of genomic regions affecting plant height in sorghum and maize. Theor Appl Genet. 90:380–388.

Poulson, M.E., M.R. Boeger, and R.A. Donahue. 2006. Response of photosynthesis to high light and drought for *Arabidopsis thaliana* grown under a UV-B enhanced light. Photosynthesis Research. 90: 79-90.

Rami, J.F., P. Dufour, G. Trouche, G. Fliedel, C. Mestres, F. Davrieux, P. Blanchard, and P. Hamon. 1998. Quantitative trait loci for grain quality, productivity, morphological and agronomical traits in sorghum (*Sorghum bicolor* L. Moench). Theor Appl Genet. 97:605–616.

Raven, J.A. 2002. Selection pressures on stomatal evolution. New Phytologist. 153:371-386.

Rooney, W.L. 2004. Sorghum improvement—integrating traditional and new technology to produce improved genotypes. Advances in Agronomy. 83:37–109.

Rooney, W.L., J. Blumenthal, B. Bean, and J.E. Mullet. 2007. Designing sorghum as a dedicated bioenergy feedstock. Biofuels, Bioproducts and Biorefinig. 1(2):147 – 157.

Rosenow, D.T. 1987. Breeding sorghum for drought resistance. *In*: J.M. Menyonga, T. Bezune and A. Yuodeowei (Eds.) Proceedings of the International Drought Symposium. OAU/STRCSAFGRAD Coordination Office, Ouagadougou, Burkina Faso, p. 19–23.

Rosenow, D.T. 1993. Breeding for lodging resistance in sorghum. *In*: Proc 18th Biennial Grain Sorghum Res, Feb 28-Mar 2, 1993, Lubbock, TX, p. 122-126.

Rosenow, D.T. and L.E. Clark. 1981. Drought tolerance in sorghum. *In*: Proc 36th Annual Corn and Sorghum Res Conf, December 9-11, 1981, Chicago, IL, p. 18-30.

Rosenow, D.T., and L.E. Clark. 1995. Drought and lodging resistance for a quality sorghum crop. *In*: Proc 50th Ann Corn and Sorghum Industry Res Conf, Dec 6 – 7, 1995, Chicago, IL, American Seed Trade Association, p. 82-97.

Rosenow, D.T., and J.A. Dahlberg. 2000. Collection, conservation and utilization of sorghum. p. 305–328. *In* C.W. Smith and R.A. Frederiksen (ed.) Sorghum: Origin, history, technology, and production. John Wiley & Sons, New York.

Rosenow, D.T., G. Ejeta, L.E. Clark, M.L. Gilbert, R.G. Henzell, A.K. Borell, and R.C. Muchow. 1996. Breeding for pre- and post-flowering drought stress resistance in sorghum. *In*: Proc Int Conf on Genetic Improvement of Sorghum and Pearl Millet, Sept 23–27, 1996, Lubbock, TX, p. 400–411.

Rosenow, D.T., J.E. Quisenberry, C.W. Wendt, and L.E. Clark. 1983. Drought tolerant sorghum and cotton germplasm. Agric.Water Manage. 7:207-222.

Sanchez,A.C., P.K. Subudhi, D.T. Rosenow, and H.T. Nguyen, 2002. Mapping QTLs associated with drought resistance in sorghum (*Sorghum bicolor* L. Moench). Plant Molecular Biology. 48: 713–726.

Sandhu, D., J.A. Champoux, S.N. Bondareva, K.S. Gill. 2001. Identification and physical localization of useful genes and markers to a major gene-rich region on wheat group 1S chromosomes. Genetics. 157:1735–1747.

Squire, G.R. 1993. The physiology of tropical crop production.CAB Int., Wallingford, UK.

Srinivas, G., K. Satish, R. Madhusudhana, R. Nagaraja Reddy, S. Murali Mohan, and N. Seetharama. 2009. Identification of quantitative trait loci for agronomically important traits and their association with genic-microsatellite markers in sorghum. Theor Appl Genet. 118: 1439 – 1454.

Subudhi,P.K., and H. T. Nguyen, 2000. Linkage group alignment of sorghum RFLP maps using a RIL mapping population. Genome. 43: 240–249.

Tanksley, S.D. 1993. Mapping polygenes. Annu. Rev. Genet. 27: 205-233.

Tuinstra,M.R., E. M. Grote, P. B. Goldsbrough, and G. Ejeta, 1996. Identification of quantitative trait loci associated with pre-flowering drought tolerance in sorghum. Crop Sci. 36:1337-1344.

Tuinstra, M.R., E.M. Grote, P.B. Goldsbrough, and G. Ejeta. 1997. Genetic analysis of post-flowering drought tolerance and components of grain development in *Sorghum bicolor* (L.) Moench. Mol Breed. 3:439–448.

Tuinstra, M.R., G. Ejeta, and P.B. Goldsbrough. 1998. Evaluation of near-isogenic sorghum lines contrasting for QTL markers associated with drought tolerance. Crop Sci. 38:835–842.

UNESCO (United Nations Educational, Scientific and Cultural Organizations). 1998. A summary of the monograph. *In:* World water resources, A new appraisal and assessment for the 21st century. RPL Design, UK.

United Nations. 2011. World population to reach 9.1 billion in 2050, UN projects. UN news centre. Available at http://www.un.org/news/ (Verified 27th April 2011).

USDA (United States Department of Agriculture). 2010. World agricultural production. Foreign Agricultural Service / USDA Office of Global Analysis. National Technical Information Service. Washington, DC.

Veldboom, L.R., M. Lee, and W.L. Woodman. 1994. Molecular facilitated studies of morphological traits in an elite maize population. II. Determination of QTLs for grain yield and yield components. Theor Appl Genet. 89:451–458.

Voorrips, R.E. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. The Journal of Heredity. 93 (1): 77-78.

Wang, S., C.J. Basten, and Z.B. Zeng. 2007. Windows QTL cartographer 2.5. Available at http://statgen.ncsu.edu/qtlcart/WQTLCart.htm (verified 7th Jan, 2010). Dept. of Statistics, North Carolina State University, Raleigh.

Wright, G.C., R.C.N. Rao, and G.D. Farquar. 1994. Water-use-efficiency and carbon isotope discrimination in peanuts under water deficit conditions. Crop Sci, 34: 92-97.

Xiao, J., J. Li, and S.D. Tanksley. 1996. Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a sub-specific rice cross. Theor Appl Genet. 92:230–244.

Xin, Z., R. Aiken, and J. Burke. 2009. Genetic diversity of transpiration efficiency in sorghum. Field Crops Research. 111:74–80.

Xu, W., P.K. Subudhi, O.R. Crasta, D.T. Rosenow, J.E. Mullet, and H.T. Nguyen. 2000. Molecular mapping of QTLs conferring stay green in sorghum. Genome. 43:461–469.

Yang, W., A. Nadolska-Orczyk, K.C. Wood, D.T. Hahn, P.J. Rich, A.J. Wood, H. Saneoka, G.S. Premachandra, C.C. Bonham, J.C. Rhodes, R.J. Joly, Y. Samaras, P.B. Goldsbrough, and D.Rhodes, 1995. Near-isogenic lines of maize differing for glycinebetaine. Plant Physiol. 107: 621-630.

VITA

Name:	Mohankumar Heraganahally Kapanigowda
Address:	Sorghum Breeding and Genetics, Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843-2474
Email Address:	mohangowda_hk@yahoo.com
Education:	B.S., Agriculture, University of Agricultural Sciences, 2000 M.S., Agronomy, University of Agricultural Sciences, 2002 Ph.D. Plant Breeding, Texas A&M University, 2011