

DETERMINING GENETIC OVERLAP BETWEEN STAYGREEN, LEAF WAX AND CANOPY  
TEMPERATURE DEPRESSION IN SORGHUM RILS

A Thesis

by

HENRY OCHIENG AWIKA

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

MASTER OF SCIENCE

May 2012

Major Subject: Plant Breeding

Determining Genetic Overlap between Staygreen, Leaf Wax and Canopy Temperature

Depression in Sorghum RILS

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May 2012

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## ABSTRACT

Determining Genetic Overlap between Staygreen, Leaf Wax and Canopy Temperature Depression in Sorghum RILS. May 2012

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Chair of Advisory Committee: Dr. Dirk Hays

Crops adapted to dry conditions are essential to meet future food, feed and energy needs. Knowledge of interaction between drought tolerance traits and their response to varying water supply conditions would improve selection for yield stability traits. This study focused on determining the association between the QTL regulating the staygreen trait in sorghum with improved canopy temperature depression (CTD) as regulated by total and compositional epicuticular wax content in a recombinant inbred line population derived from BTx642 and RTx7000. Phenotypic data were collected in 3 replicated field trials and 1 greenhouse trial. Plants with higher leaf EWL had cooler canopies. Our results also confirmed that staygreen genotypes are able to maintain cooler canopy than the non-stay-green genotypes under drought and hot conditions. We have suggested that wax might offer a more stable indicator for selection of drought tolerance under a variety of weather conditions and across different environments. Composite interval mapping identified a total of 28 QTL, fifteen of which had significant overlaps. The overlap between QTL for cuticular leaf wax and QTL for staygreen exhibits a departure from the QTL overlaps for other traits with that of cuticular leaf wax. We have also suggested that under drought stress, the QTL for staygreen may be expressed earlier in time (at anthesis) than had been previously believed.

## DEDICATION

To my shy but cheerful, little daughter, Miriro

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I finally thank my brothers, Tom and Joseph for their kindness and background support.

## NOMENCLATURE

ANOVA	Analysis of variance
CTD	Canopy temperature depression
EWL	Epicuticular wax load
DG	Digital genotyping
DTF	Days to flowering
FT	Flowering time
LAUD	Leaf area under decline
LOD	Log of odds
LR	Likelihood ratio
LSD	least square difference
LSMean	Least square mean
QTL	Quantitative trait locus
RFLP	Restriction fragment length polymorphism
SS	Sum of squares
SSR	Single sequence repeats
Stg	Staygreen
TD	Temperature depression

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## CHAPTER I

### INTRODUCTION AND LITERATURE REVIEW

The world population is widely projected to hit 9 billion by the year 2050, with a corresponding need to double the world crop yields to meet the demand by the same year (Rothstein, 2007). Sorghum provides a critical food supply for over 30 countries in the Tropics and is the world's fifth most important food crop after wheat, maize, rice, and potatoes. Sorghum is also a vital source of livestock feed and bioethanol fuel in the U.S where 80% is non-irrigated (Ayeneh, 2002; Blum, 2005; Singh, 2006). Energy conversion and beverage demand from traditional and industrial breweries are quickly gaining prominence (Rooney, 2007; Saballos, 2008). Sorghum becomes of more critical value when entire regions, especially in Africa and Asia depend on it as human food. Any aspect threatening sorghum productivity is putting entire livelihoods at risk.

The increased food needs faces stiff challenge from increasing frequencies of abiotic stresses associated with Global Warming. These stresses such as heat and drought are responsible for severe yield losses and the issue has become increasingly urgent with the reduction in arable land. Crops that are able to remain productive under such conditions will remain crucial in meeting world food, fodder and fiber needs. Such plants should be able to photosynthesize and accumulate sufficient biomass which can be exploited. Carbohydrate ( $\text{CH}_2\text{O}$ ) constitutes about 90% of plant biomass (Ruan, 2010) and is an important yield determinant. Studies have shown that stress induces inhibition to biomass accumulation. Sorghum is a model drought tolerant crop among the domesticated grasses (Kumari, 2007). To enable sorghum to continue coping with the ever reducing natural water supply, enhanced discovery, understanding and manipulation of traits that sustain yield in water-stressed conditions becomes

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This thesis follows the style of Crop Science.

imperative. This study explores the genetic association between leaf epicuticular wax and the stay green traits in sorghum and their influence on leaf cooling.

Wax has been linked to adaptive mechanism of coping with drought (Jordan, 1984). Other recent studies of stay green (stg) QTL stg1, stg2, stg3, stg4 have confirmed their close association with leaf photosynthetic stability during post-grain setting in sorghum (Crasta, 1999; Ejeta, 2007; Haussmann, 2002; Xu, 2000). Canopy temperature depression (CTD) has been shown to be one of the predictive indicators of physiological water status in a plant (Ayeneh, 2002; Fan T., 2005). Balota (2007) showed that CTD also positively correlated with crop yield in non-irrigated water stressed wheat fields, while a negative relationship has been reported in a genotype adapted to irrigated conditions (Keener, 1983). Studies in wheat have also shown that CTD is independent of individual organ temperature depressions (TD), and that the leaf TD strongly correlates positively with CTD under all growth conditions (Ayeneh, 2002).

The stay green trait has also been shown to correlate positively with CTD and yield and thereby suggested both can be used to select for drought tolerance (Kumari, 2007). However, since similar basic responses to drought-related stress have been reported in all crops (Blum, 2005) the assumption in this study is that epicuticular wax load (EWL) and stg traits are associated. It has been shown that the plant wax accumulation at flowering is responsive to water treatment and confers a lower canopy temperature hence higher CTD. Past studies have not clearly shown the association of EWL and the stg traits and influence on CTD. Understanding this association could reveal a mechanism for staygreen that could be used in further selection for drought tolerant lines. This study fills this gap and *determines the genetic overlap between leaf epicuticular wax, canopy temperature depression, and the stay green trait in sorghum RIL population*. The sorghum recombinant inbred lines (RILs) population used in this study were derived from a cross between RTx7000 (a pre-flowering drought tolerant,

but post-anthesis susceptible) and, BTx642 (a post-anthesis stay green)(Van Oosterom, 1996).

The following review of discusses the interaction of drought stress and plant response, focusing on staygreen and leaf cuticular wax traits, and canopy temperature depression.

### **1.1 General water and temperature scenario**

Temperatures higher than those required for plant growth may lead to delayed anthesis in some sorghum genotypes (Rosenthal, 1991). On the other hand, it has been shown that temperature extremes just before anthesis often result in floret abortion, reduced seed set and lower grain yield (Hays, 2007; Rosenthal, 1991). High temperatures under water stress also influence flowering duration - FD (time required for tip to basal anthesis within a grain sorghum panicle) and flowering period – FP (time required for tip to basal anthesis within a field) (Jordan, 1984; Walulu R. S., 1994). Rosenthal<sup>16</sup> showed that scarcity of water at anthesis lead to remarkable yield reduction or crop failure in grain sorghum [*Sorghum bicolor* (L.) Moench]. The same study further asserts how rainfall is difficult to predict and that it is impractical to substantially alter the timing and amount of water stored in the soil. This becomes of even greater significance considering regions of the world dependent on rain fed agriculture.

### **1.2 Wax accumulation as drought resistance strategy**

Epicuticular wax level (EWL) along with other leaf parameters correlates positively with cell membrane stability (CMS) (Hays, 2007). CMS has been found to increase with increase in water deficit in a study of some sorghum cultivars (Ibrahim, 2001; Premachandra, 1992). As water scarcity increases so does the level of leaf and stem EWL which reduce cuticular conductance and improve plant water conservation (Tao, 2000). Epicuticular wax (EW) also moderates the net insulation on the plant leaf and

this has been known to reduce excessive water loss during low soil moisture (Morison, 2008). This fact is supported by Bengtson (Bengtson, 1978) in a study of oat where unstressed seedlings of drought resistant variety showed the highest cuticular transpiration rate, but showed the most strongly reduced transpiration rate after stress treatment; at the same time it showed the largest increase in amount of epicuticular leaf wax of the tested varieties. Sorghum phenotypes across all three isogenic lines (bloomless, the bloom type and sparse bloom) including associated hybrid types showed a negative qualitative relationship with cuticular transpiration rate, within the EWL range of 0.1 to 0.03g/sq. m (1mg to 0.3mg/sq. dm<sup>2</sup>); under most conditions this response tends to stabilize at about 0.065g per sq. meter with no more cuticular transpiration (Jordan, 1984). The bloom type seems least consistent in clearly exhibiting this kind of relationship (Ebercon, 1977). This study will show the quantitative variation of leaf wax with different water treatment regimes at three field locations. The high wax on the other hand has been shown to play a role in water and heat stress tolerance at anthesis, when the leaf EWL has been shown to peak (Blum, 2005; Sanchez, 2002). Determining EWL among the study RILs is therefore vital in discriminating variations in drought resistance.

### **1.3 Stay green and leaf wax**

The ability of sorghum to stay alive as a perennial crop under scarce water conditions is remarkable, but just like any other crop, this ability can be greatly compromised at anthesis and early grain filling stages as a result of the early onset of senescence (Kassahun, 2010; Walulu R. S., 1994). The same study contend that the ability of *Sorghum bicolor* (L.)–Moench to stay green confers delayed senescence. This phenomenon has recently been shown to correspond to stable QTL across varied environments (Tao, 2000). But it had earlier been postulated in past studies that this trait is controlled by a major gene that exhibit varying degrees of dominance in different environments (Walulu R. S., 1994). While sorghum generally dies as a result of

drought stress during grain filling, the stability of the plants with stay green traits to continue to fill grain normally has been demonstrated (Rosenthal, 1991; Xu, 2000). According to current knowledge, this is the fundamental ability of some sorghum genotypes to stay green during post-anthesis water stress (Blum, 2005; Tao, 2000). Considering the positive relationship between increased air (external) temperature and water loss, the role EWL plays in reducing cuticular transpiration becomes a critical phenotypic consideration in sorghum productivity. Both wax and stay green traits have therefore been demonstrated to play key role in sorghum grain yield parameters under water-scarce conditions.

#### **1.4 Significance and rational**

The capability of a plant leaf to sustain a healthy internal temperature in the face of external erratic fluctuations is vital in maintaining leaf cell water balance necessary for optimal flowering and seed development, and biomass accumulation (Kumari, 2007; McLaughlin, 2004). High temperature has been shown to increase water loss from the leaves. Adequate water availability and slowed post-anthesis senescence have been shown to have a direct positive impact on yield. Leaf epicuticular wax has also been shown to deter excessive water loss through the leaf cuticle under elevated temperatures (Clarke, 1988) (Clarke, 1991) . However, the variations in canopy temperature depression (CTD) among sorghum lines and the genetic interplay with CTD, total epicuticular wax load and stay green traits have not been fully understood. It is also important to determine if leaf reflectance associated with high leaf cuticular wax confers a corresponding lower leaf internal temperature under dry and watered conditions. And whether does the Stg gene in some sorghum genotypes overlap with genes for high leaf wax. If this genetic overlap exists, then it would be expected that this relationship may play a role in CTD regulation. This area of enquiry has not been fully explored, and forms the emphasis of this study. Looking at the genetic overlap between the stay green QTL and QTL for high leaf wax and their relationship with CTD



will be important for breeding sorghum cultivars adaptable to dwindling soil moisture in many parts of the tropics. The elucidation of the genetic association will open fresh frontiers for targeted trait selection in sorghum breeding. Dissecting the genomic positions and overlaps of major QTL for stg and EWL will also broaden the hunt for candidate genes controlling these biochemical processes.

### 1.5 Study objectives

The *long-term goal* is to contribute to the pool of knowledge of mechanisms associated with drought tolerance traits. Elucidating the association of plant drought traits behind specific QTLs provides a niche for marker-assisted selection of the desired trait. The *objective of this study* is to map and analyze the QTL for stay green and QTL for leaf wax in order to establish their association with canopy temperature depression. This will give additional valuable insight into the genetic regulation of cuticular leaf cooling mechanisms that is vital in reducing excessive water loss. *The central hypothesis* is that the canopy temperature depression is gene-regulated and is associated with overlapping QTL for both stay green and high leaf wax.

The following *specific aims* will be used to test the hypotheses.

***Objective 1: Establish the relative genotype, environments and GxE interaction on association between EWL and CTD and its variability due to location.***

The hypothesis to be tested is that there exist locational variations in CTD corresponding to EWL and water treatment regime. The expected outcome is that the relationship between EWL and CTD will be determined. This will provide a vital selection indicator of lines with greater promise and adaptability to changing temperature and water regimes, and for manipulating gene regulating leaf wax to improve on drought tolerance.

***Objective 2: Determine the association between QTL for stay green and leaf wax and their relationship with CTD among the RILs.***

The hypothesis to be investigated is that stay green and leaf wax QTL loci have a co-location with CTD. The main output expected is that the association between stay green and leaf wax QTL and CTD will be determined. This will provide a crucial basis for selecting cultivars with overlapping QTL active in controlling CTD.

## CHAPTER II

### EFFECT OF EPICUTICULAR WAX LOAD ON CANOPY TEMPERATURE DEPRESSION AND ITS VARIABILITY DUE TO LOCATION

#### **2.1 Introduction**

At a scale of 1.54 billion ha of arable land, agricultural crop activity dominates the use of freshwater and accounts for some 70% of withdrawals from water resources globally (FAOSTAT, 2007). Such large extractions of water has led to huge reductions in river flow, sometimes, to long and repeated periods of zero flow for several major river systems (Ma, 2003). As most of the water withdrawn by agriculture is lost in evaporation and transpiration (Kemanian, 2005), in marked contrast to domestic and industrial withdrawals, agriculture accounts for 80–90% of freshwater used by humans (Shiklomanov, 2003). Drought is expected to limit productivity of more than half of the earth's arable land in the next 50 years (Solomon, 2007), while the world population is expected to clock the 9 billion mark by the year 2050 (FAOSTAT, 2007). Clearly, making agriculture sustainable requires a major reduction in water demand by crops for food, bioenergy and feed. Enhanced manipulation and selection for drought resistance therefore becomes imperative. From the water loss perspective, high leaf wax has been positively correlated with a reduced transpiration rate under low soil water. This chapter explores the relative effects of genotype, environment and genotype X environment interactions on variability of leaf wax and its association with increased canopy temperature depression (CTD) or cooler leaves. This will be important in screening for cultivars adaptable to drought environments by combining low transpiration and self-cooling under varying temperature regime.

## **2.2 Materials and methods**

### **2.2.1 *Germplasm***

One hundred F<sub>12</sub> recombinant inbred lines (RILs) developed from a cross of BTX642 and TX7000 were used. BTX642, formerly referred to as B35, has been as a useful source staygreen for research and development of drought resistant commercial hybrids (Harris, 2007; Tao, 2000). It is an inbred BC<sub>1</sub> derivative line of IS12555 durra sorghum from Ethiopia, and varying dominance is exhibited by its genes conferring the staygreen trait (Sanchez, 2002; Walulu R. S., 1994; Xu, 2000). It is susceptible to pre-flowering drought stress but highly resistant to post-flowering drought stress (staygreen trait) and has relatively low yield potential. The TX7000 is an elite line released and distributed in the 1940s as 'Caprock' open pollinated, grain sorghum variety, by Texas Agricultural Experiment Station at Lubbock. It was derived from a 'Kafir' x 'Milo' cross, and was later used as a male parent in the late 1950s (Xu, 2000). It is currently public line used to develop sorghum hybrids in the United States (Subudhi, 2000). It has high yield potential, and is pre-flowering drought resistant but is drought susceptible at post-flowering (Xu, 2000).

### **2.2.2 *Evaluation environments***

A total of four environments were used. Three field experimental locations to characterize the EWL were located at Texas A&M AgriLife field stations in College Station (irrigated), Weslaco (irrigated) and Corpus Christi (drought). Land preparation was done uniformly using standard implement plowing at depth 20.0~24.0 cm, followed by rolled beds a day before planting and subsequent sweep and rolled cultivations after germination. Two replications of 100 Tx642/Tx7000 RILs and the parental lines were nested in each location. Weslaco was dry planted February 16, Corpus Christi on March 9 and College Station March 23. All plots were in randomized complete block design (RCBD). Spacing between rows was 1 m with each row representing one RIL was 5.5 m long. Flood irrigation was applied in Weslaco and

College Station at days 1, 30 and 74 after planting. Complete seedling emergence was 7 days after planting in all locations and for all the lines. Canopy temperature readings and leaf samples for wax analysis were taken at 50% flowering observed independently in each row.

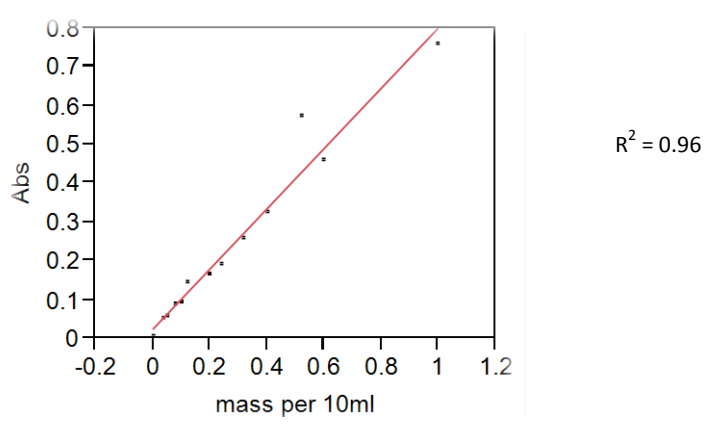
The 100 RILs were dry planted with three plants in two replications in 12 inch diameter pots filled with “Metromix” forest soil mixture. Watering occurred 1 day after planting and at subsequent intervals of 4 to 5 days thereafter until flowering. Two dry days were allowed before each sampling date. Temperatures in the chamber were kept at between 26 and 31<sup>0</sup>C. Canopy temperature readings and leaf samples for wax analysis were taken on the same day at 50% anthesis.

### ***2.2.3 Phenotypic evaluation***

Two plants per RIL at 50% anthesis, in each location were randomly selected and 10 fresh leaf disks (7 mm diameter) were obtained from the flag leaf of each of the plants, separately into clean vials. Colorimetric method developed by Ebercon et al (1977) was used to extract the leaf cuticular wax and to determine the wax load in mg·dm<sup>-2</sup>. Wax was extracted from the disks by covering each sample with 1.5ml redistilled chloroform for 30s. The resulting suspension was transferred into clean 2.0ml vials using a glass pipette. After completely evaporating the chloroform in a ventilation hood for 12hrs, reaction acidified potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) was used to produce desired color change. The reagent was prepared by adding 1000 ml of H<sub>2</sub>SO<sub>4</sub> to 20g powdered K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and 40ml deionized water. The mixture was heated, while stirring vigorously, to below boiling point until the solution became clear. To each of the 2.0ml vial containing dried wax, 0.5ml of the reaction mixture was transferred and the content thoroughly mixed on a vortex mixer, then heated for 30min in boiling water bath. The sample solutions were left to cool and 1.5ml dH<sub>2</sub>O added to each sample. The reaction was left to cool and color change to develop. Absorbance values were determined from

the average of two readings at  $A_{590}$ , and values converted to  $\text{mg}/\text{m}^2$  using Beer-Lambert formula (Ebercon, 1977)

A standard curve (Fig. 1) was generated from carnauba wax known to have a strong positive correlation with sorghum wax. A 40mg pure wax sample was used to prepare 12 different known concentrations of standard solution following Ebercon et al (1977). Using Beer-Lambert formula, the gradient of regression fit line was used as the constant extinction coefficient ( $\epsilon$ ), in determining the unknown concentrations of the samples.



**Fig 1.** Calibration curve using a series of 12 standard solutions were used to prepare calibration curve. On the Y-axis are the varying masses of pure wax in mg dissolved in 10ml of solution. Y-axis are the respective absorbance values, which increased with increase in wax concentration.

Concentration of wax as predictor of absorbance was found to be significant ( $P < .0001$ ) at 95% confidence level.

Extinction coefficient,  $\epsilon$  (slope)  $= A/C = 0.78$ , where, A = absorbance values of standard solutions; C = wax load per 10ml solution. The  $\epsilon$  constant was used to determine the wax loads (EWL) per square decimeter of sample leaf area:

$EWL = UA_{590}/\varepsilon$  for 10 disks with area  $3.85\text{cm}^2$ . For  $1\text{dm}^2 = EWL*100/3.85$ , where:  $UA_{590}$  = absorbance of sample (unknown optical density).

#### **2.2.4 Canopy temperature depression**

Temperature was taken from the flag leaves between 11.45 am and 1.45pm when correlation of CTD at anthesis and yield is highest (Balota, 2007) and plant water stress is expected to be high (Fan T., 2005). The CTD was measured using a hand-held IRT (model OAKTon Pro, Class 2(ii) Laser Product, Output Wavelength 630 – 670nm) with external probe was used. The incident laser point was beamed at  $45^\circ$  tilt to the horizontal and from 5 inches perpendicular to the contact flag leaf. The external probe was simultaneously used to record the ambient temperature. The difference was taken as the temperature depression. The values of the running checks provided a basis for assessing site variability.

#### **2.2.5 Data analysis**

Data was analyzed using SAS JMP

(<http://www.sas.com/search/searchquery.html?Find=Search&qt=jmp&q=-url%3A%2Foffices%2F&qc=extsas>) software. Using ANOVA, correlation and regression analyses, individual and combined were determined for the 3 field locations..

Differences in daily external temperature on certain sampling days were noted to be significant. Corresponding CTD were moderated by percentage value of daily means for each subject location following recommendations similar recommendations (Reynolds, 1994; Reynolds, 1998), (Wilman, 1992) and (Keener, 1983). The temperature depression data were clustered on range-basis of  $0.5^\circ\text{C}$  difference and temperature depression of the genotypes calculated as a percent of the mean temperature of the cluster. This provided a reduction of variation due to transient external temperature changes within the time window of data collection. This transformed data also correlated positively ( $r=0.94$ ) with the row data, and has been applied in regression

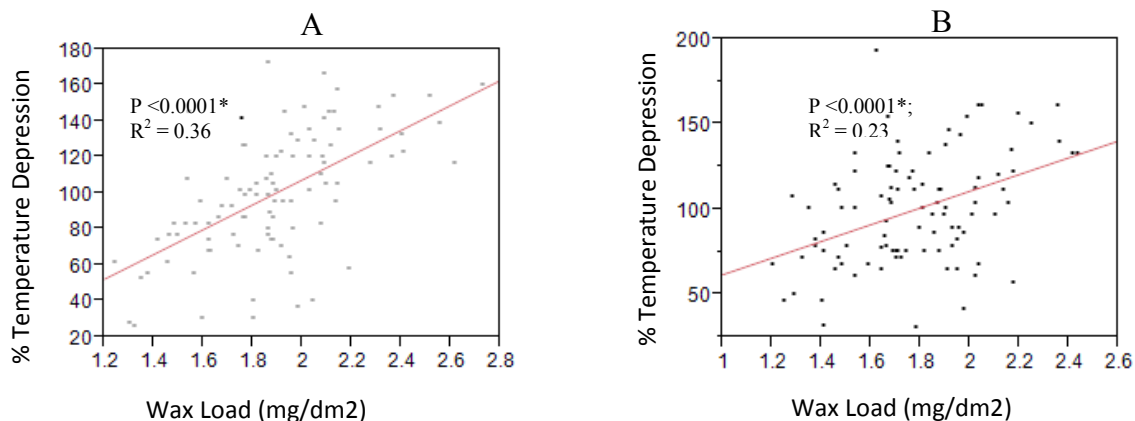
analyses. Log-transform values of cuticular wax load and raw data for flowering time were used in correlation and regression.

Homogeneity of variance among locations was determined using Lavene Test as modified by Brown-Forsythe (Ott, 2010). Normality assumption was checked from Shapiro-Wilk Test (Ott, 2010). Correlations were used for checking linear relationship and regression procedures were applied to determine CTD variation explained by the model, and significance of EWL and locational effect on CTD. Mean differences were determined using Fisher's LSD (least square difference). Relevant statistical assumptions were considered, in arriving at the inferences.

## 2.3 Results and discussion

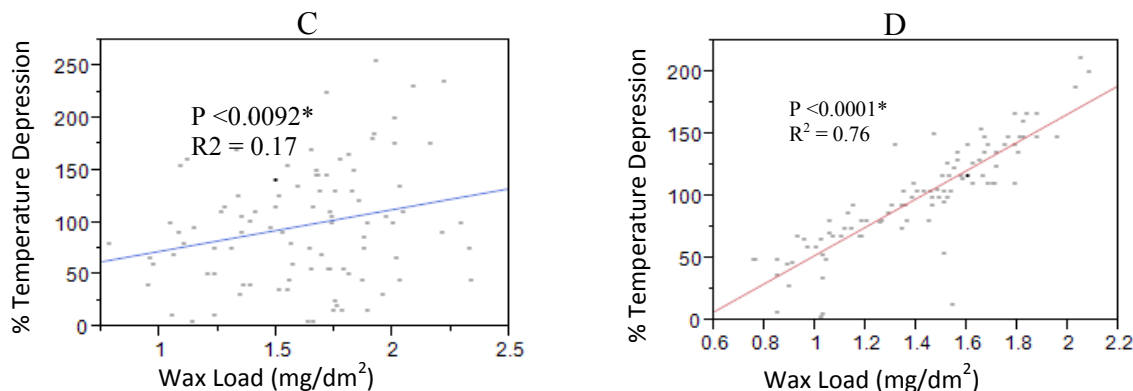
### 2.3.1 Variation of TD against wax among the field locations

Percent leaf temperature depression (TD) was regressed against leaf log of leaf wax (Figure 2). Among the field locations, the relationship of TD with wax was strongest in Corpus Christi (drought environment) than in College Station and Weslaco (irrigated environments). The percent variation of TD explained was 36% at Corpus Christi followed by College Station at 23% and Weslaco 17%.



**Fig. 2:** Regression relationship of temperature depression (TD) and wax load (EWL) in each location. X-axis values are log transformed EWL which correlate strongly with the row EWL values. Y-axis are TD as a percent of clustered measurement day mean. A, represents Corpus Christi, B, College Station, C, Weslaco and D, greenhouse.





**Fig. 2:** Continued.

### 2.3.2 Combined analysis

The multi-environment trial analysis obtained the estimates of effects on the phenotype due to the genotype, environment and their interaction between genotype and environment. The variation of leaf wax and corresponding temperature depression were significantly affected by the genotype (G) and environment (E). The G×E interaction was significant for temperature depression (CTD) but not for leaf wax. This shows that the genotypes are likely to have greater variation across different environments than leaf wax. The G×E interaction therefore has more confounding effect on CTD across environments, than on leaf wax. CTD may be less valuable predictor of yield parameters than wax across many environments.

**Table 1:** Combined analysis for all locations. Genotype (G) and environment (E) effect on leaf epicuticular wax (EWL), canopy temperature depression (CTD) and days to flowering (DTF). The

Source	DF	EWL		CTD		DTF	
		F Ratio	Prob > F	F Ratio	Prob > F	F Ratio	Prob > F
Environment	3	139.6	<.0001*	238.5	<.0001*	11.52	0.0419*
Genotype	99	145.3	<.0001*	83.6	<.0001*	83.7	<.0001*
G x E	297	7.6	0.1833	56.5	0.0002*	0.74	0.5291

### 2.3.2 Correlation analysis of the phenotypes within and across location

The results of correlation analyses among the phenotypic traits in all the locations are summarized in Table 1.

**Table 2:** Phenotypic correlations between leaf wax (EWL), temperature depression (CTD) and day to flowering (FT)<sup>#</sup> in all locations<sup>β</sup> for all RIL

	gh-CTD	gh-EWL	gh-FT	cs-CTD	cs-EWL	cs-FT	wl-CTD	wl-EWL	wl-FT	cx-CTD	cx-EWL	cx-FT
gh-CTD	1.00	0.86*	-0.09	0.30*	0.58*	0.22*	0.04	0.80*	-0.12	0.28*	0.40*	-0.10
gh-EWL		1.00	-0.04	0.38*	0.65*	0.12	0.19	0.87*	-0.02	0.24*	0.49*	-0.07
gh-FT			1.00	-0.10	-0.06	0.07	-0.24*	-0.01	0.39*	0.25*	0.06	0.53*
cs-CTD				1.00	0.44*	-0.08	0.10	0.48*	-0.21	0.29*	0.30*	0.10
cs-EWL					1.00	0.28*	0.21*	0.74*	-0.17	0.48*	0.85*	0.23*
cs-FT						1.00	-0.24*	-0.15	0.38*	-0.32	0.24*	0.27*
wl-CTD							1.00	0.18	-0.32*	0.18	0.21*	0.32*
wl-EWL								1.00	-0.07	0.35*	0.62*	-0.16
wl-FT									1.00	0.38*	-0.21*	0.60*
cx-CTD										1.00	0.57*	0.56*
cx-EWL											1.00	0.29*
cxFT												1.00

<sup>#</sup> 3 phenotypic values are represented in columns and rows

<sup>β</sup> Locations are represented by before the phenotypes (capital letters): **gh** – greenhouse; **wl** – Weslaco; **cs**- College station; **cx** – Corpus Christi

\*Significant at 0.05.

Within a location, correlation between leaf wax and TD followed closely the relationship described in regression analysis above. Among the field locations, the drought environment produced a stronger positive correlation than the irrigated. Corpus Christi was greatest (r

= 0.57), followed by College Station (0.44) and Weslaco being statistically insignificant (0.18). Greenhouse had the highest correlation ( $r = 0.86$ ). These results support the fact that amount total of leaf wax has a relationship with the ability of a plant to cool itself. Under field conditions, this relationship is much stronger during water stress than under irrigation. The results point to the confounding genotype X environment (G X E) interaction expected in the field, which was much lower under controlled environment (greenhouse). The G X E analysis is shown on Table 1

Analysis of correlation of leaf wax and TD between the field locations revealed that TD had a generally weaker correlation between locations than leaf wax. The results are condensed in Table 3, below.

Temperature depression correlations between the field locations were generally weak. Correlation between Welsaco (wl-CTD) and College Station (cs-CTD), and that between wl-CTD and Corpus Christi (cx-CTD) were not statistically significant at  $r = 0.10$  and  $0.18$ , respectively. That between cs-CTD and cx-CTD was significant ( $r = 0.29$ ). The greenhouse (gh-CTD) values correlated significantly with those of cs-CTD and cx-CTD; the correlation was not significant with wl-CTD. Correlation of wax values between field locations and the greenhouse were all statistically significant.

**Table 3:** Correlations of phenotypes between field locations (a), and between each field location and greenhouse (b). Overall the correlation is stronger for leaf wax than TD.

*a) Correlation of phenotypes between field locations*

LOCATION	<u>TD</u>	<u>EWL</u>
Weslaco - College Station	0.10	0.74*
Weslaco - Corpus Christi	0.18	0.62*
C. Christi - C. Station	0.29*	0.85*

*b) Correlation of phenotypes between greenhouse and field locations*

Greenhouse – C. Station	0.30*	0.58*
Greenhouse - Weslaco	0.04	0.80*
Greenhouse - C. Christi	0.28*	0.40*

It can be clearly observed that temperature depression (CTD) varied more among the environments than did leaf wax (EWL). These results suggest that CTD was rather unstable and the genotype X environment (G X E) interaction seems to confound this phenotype more than leaf wax. This- further confirms the results reported in the G X E analyses in Subsection 2.3.2, Table 1. The CTD may be suitable for selection only at a particular environment and under rather specific conditions within an environment. Further studies may be necessary to determine which between EWL and CTD will be better suited in selection for yield parameters within an environment. Leaf wax seems to exhibit a stable correlation across all the study environments and may be a more dependable tool for indirect selection in a variety of environments. The next section discusses the mean phenotypic values and show how the relationships outlined above reflects in their stability in different environments.

### **2.3.3 Comparing location means of phenotypes**

Mean differences at different locations were found to be significant for all the traits (leaf cuticular wax, temperature depression and the number of days to flowering) (Tables 4). Corpus Christi had consistently high wax, largest cooling effect and longest flowering time. Between the two irrigated field environments, College Station had higher values than Weslaco. Greenhouse had the lowest LSMeans.

**Table 4:** Phenotypic LSMean differences between locations. Fisher's LSD has been used to compare LSMeans (least square means) n = 100. Locations with unique letter are significantly different.

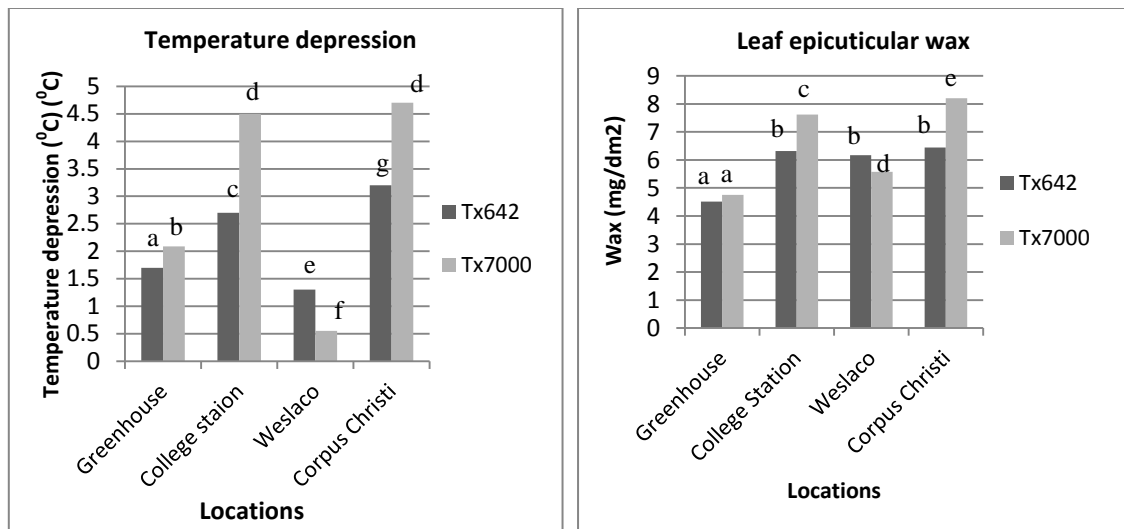
Location	EWL- LSMean	CTD-LSMean	DTF-LSMean
Corpus Christi	6.8814091 <b>a</b>	3.2240816 <b>a</b>	84.716585 <b>a</b>
College Station	6.1379592 <b>b</b>	2.7768041 <b>b</b>	81.738818 <b>b</b>
Weslaco	5.3619225 <b>c</b>	1.6260204 <b>c</b>	74.142536 <b>c</b>
Greenhouse	4.3089869 <b>d</b>	0.9917172 <b>d</b>	73.165448 <b>cd</b>

Parental means shown in Figure 3 follow closely the trend for the RIL. Tx7000 which is pre-anthesis drought tolerant, had consistently higher cuticular wax values than the Tx642 (staygreen line) in all field and greenhouse environments apart from Weslaco,

which showed the reverse for wax. There was no significant difference in days to flowering (DTF) for the Tx7000 in all the irrigated locations but the difference was significant in Corpus Christi (drought). This agrees with the observation suggesting that the plants generally took more days to flower under drought.

All the RILs showed significant differences in the trait values in all the locations. These results are summarized in the Table 4, above. Another studies have showed that (Craufurd, 1993) showed that introduction of severe water stress under high temperature at flowering and booting lead to largest reduction in grain yield among the early flowering sorghum genotypes compared to the late flowering ones but no significant difference for the vegetative crops. We have also demonstrated that high leaf wax correspond to cooler plant canopy (larger TD). The plants in drought condition also kept their canopy much cooler than the ambient air temperature, compared to those grown in watered conditions. Large canopy temperature depression been associated favorable plant leaf osmotic balance and higher grain filling duration, biomass and grain yield (Kemanian, 2005; Kumari, 2007; Richards, 2002). We conclude that high leaf wax may play an important role in preventing yield losses under low soil moisture and high temperatures among the genotypes with shorter days to flowering. Wax may also be playing important role in preventing yield losses in the later flowering genotypes.

In the combined analyses (Table 1), the G x E interaction was not statistically significant for both wax and flowering time (DTF), but was significant for temperature depression. The main effects, genotype and environment were independently significant for all the three traits. This supports our assessment in Section 2.4.3, suggesting a greater instability of CTD due to a strong G x E interaction.



**Fig. 3:** Parental mean phenotypic differences between locations. Differences are specific to the trait compared between the parents and between the locations. Values connected by the same letter for that trait are not significantly different.

Low water stress has been shown to signal plant responses leading to the accumulation of leaf wax as a water loss reduction strategy. From our results, these responses seem consistently accompanied by the ability of plant to keep itself cooler than the surrounding air temperature. Keener (1983) demonstrated three indices developed from the study of yield in an arid environment (Keener, 1983). These included canopy-air temperature differential, vapor pressure deficit and net radiation. In arid conditions with severe air water vapor deficit, each of the three indices examined had equal effect on yield. Net radiation became more important in a humid year. It is possible that the frequent transient cloudiness observed during measurement in Weslaco could have resulted in the larger confounding overall effect on the relationship between wax and TD, than in College Station or Corpus Christi, both of which had steady sunlight during measurement. Both the correlation between TD and wax ( $r=0.10$ ) and variation of TD explained ( $R^2 = 0.17$ ) were much lower in Weslaco than in College Station ( $r = 0.44$ ;  $R^2 = 0.23$ ), though both had similar water treatments. These results show that the strength of the relationship between leaf wax and TD is dependent on both soil water status and stability of other environmental conditions. More stable conditions such as greenhouse

produce a stronger correlation between the phenotypes. Less stable external environment lowered this relationship, but increased water stress led to an improved positive correlation.

Air temperature at College Station just before and during flowering was the highest in early Summer (at average of 38<sup>0</sup>C), followed by Corpus Christi (36<sup>0</sup>) and Weslaco (28<sup>0</sup>). High temperature has been associated with high wax accumulation especially around anthesis. From the study by Keener(Keener, 1983) and (Fan T., 2005), it would be expected that the high humidity at Corpus Christi should have further confounded the wax-TD relationship more than the other field locations. However, plants at Corpus Christi scored higher in wax means and the corresponding correlation with CTD than College Station. Epicuticular leaf wax in the periods close to anthesis may be more a factor of plant response to low soil water than high temperature per se. Wax also seems to play a more stable role in controlling CTD under water stress than high temperature does.

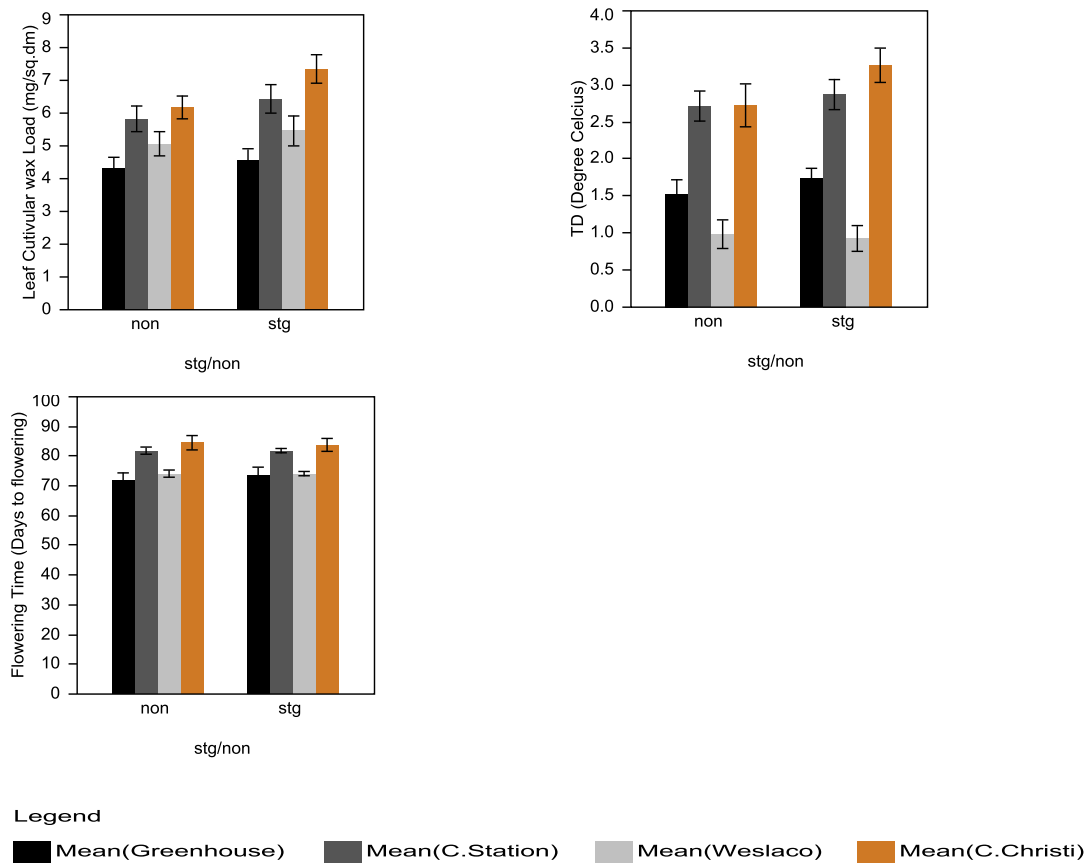
The CTD seemed to follow in tandem correlation with wax even though air temperature influenced CTD's irregular fluctuations. Greater CTD has been attributed to increased stomatal conductance and crop water use, under favorable soil-water conditions (Amani, 1996; Hays, 2007). During all stages of plant development from seed emergence, CTD correlates strongly with yield (Balota, 2007; Blum, 2005; Greenwood, 2006), and therefore has been favorably used as a selection criterion for yield under drought and heat stress. Past studies have also strongly suggested the suitability of CTD as an indicator of yield and stress tolerance must be determined for individual environments(Balota, 2007; Blum, 2005). Our study confirmed that CTD may also be very unstable and hence a less effective indicator under high soil moisture and transient weather conditions. We have shown that notwithstanding other confounding environmental factors, leaf wax has an important bearing on CTD under controlled, highly watered condition (greenhouse), normally irrigated field conditions (Weslaco

and College Station) and severe water deficit field condition (Corpus Christi). Wax might therefore offer a more stable indicator for selection of drought tolerance under a variety of weather conditions. This will be important as the world enters periods of increasingly erratic weather patterns.

#### ***2.3.5 Comparing phenotypic means of staygreen and non-staygreen RILs***

Mean cuticular wax load did not differ significantly between stay green lines and non-stay green lines within irrigated field locations and the greenhouse. There was a significant difference within the drought location. This could be a indicator to the role of both staygreen and leaf wax traits play in drought tolerance. A strong correlation between staygreen and canopy temperature depression was demonstrated by Kumari, et al (Kumari, 2007). Figure 4 shows consistently significant mean difference between staygreen and non-staygreen lines for both leaf wax and TD in the drought environment (Corpus Christi). The difference between these two genotypes was not significant in the irrigated environments. Both leaf wax and staygreen could also be used in selection for drought tolerance.





**Fig. 4:** Comparing means performance of stay green and non-stay green lines among the RILs

## 2.4 Conclusion

The study has dissected different comparisons of leaf wax and canopy temperature depression as drought tolerance parameters under the watered and dry conditions. The positive correlation between leaf epicuticular wax load (EWL) and canopy temperature depression (CTD) was stronger in drought than irrigated field conditions. Plants with higher leaf EWL have cooler canopies; as such their liking transpirational demand is also lower. Our results also confirmed that staygreen genotypes are able to maintain cooler canopy than the non-stay-green genotypes under drought dry and hot conditions. We have related our results and those based on previous studies to infer that high leaf wax

may play important role in preventing yield losses in low soil water and high temperature among the genotypes with shorter days to flowering. Finally we have suggested that wax might offer a more stable indicator for selection of drought tolerance under a variety of weather conditions.

## CHAPTER III

### QTL MAPPING OF LEAF WAX LOAD AND CANOPY TEMPERATURE DEPRESSION AND THEIR ASSOCIATION WITH QTL FOR STAYGREEN

#### 3.1 Introduction

The harvested part of part of plant seed perpetuates the continuity of agriculture. The production of that seed is dependent on healthy plant-water relations. Drought and heat stress are known to result in embryo abortion and inadequate development of the seed. Drought is therefore a major limiting factor in crop productivity. Plants have evolved the ability to cope with or escape these stress effects. In the previous chapter, we have demonstrated the phenotypic relationships between leaf wax and temperature depression as some of the drought tolerance traits. We have also reported the association between staygreen and non-staygreen with leaf wax and CTD traits among Tx642/Tx7000 RILs. Staygreen is a the ability of a plant to maintain active photosynthetic processes at maturity and this has been scored as a leaf death count (Xu, 2000) and more recently, LAUD (leaf area under decline) (Kumari, 2007). The staygreen trait has therefore been an important component in studies in terminal drought stress in sorghum (Haussmann, 2002; Kassahun, 2010). The association between QTL regulating staygreen and leaf wax and CTD will provide a crucial basis for selecting cultivars with overlapping QTL active in controlling excessive water loss.

#### 3.2 Experimental design

##### 3.2.1 Mapping population

Of the 100  $F_{12}$  RILs, 90 members of BTx642/RTx700 sorghum population were used in QTL analysis. The distribution of the whole population corresponded very closely to the normal distribution of the transformed data for 100 RILs used in the phenotypic analyses. The 90 individuals therefore were taken as representative of the whole experimental population.

### **3.2.2 QTL mapping and analysis**

Average phenotypic values per RIL were obtained across the plots per location. Four component files were used to construct the QTL mapping file. The first file was the list of linkage group and number of markers per linkage group. Name and order of the markers per linkage group were organized into the second file. Third file consisted of the genetic position in centimorgan (cM) of each marker per linkage group; this file was constructed using , MapMaker/EXP version 3.0 (Stephens, 1993). The fourth file contained genotypic data per locus and corresponding phenotypic data (EWL, CTD) per RIL average. The genotype data used had earlier been generated using digital genotyping (DG) technology that uses high throughput sequencing by Illumina Genome Analyzer Platform II. This had better resolution over the techniques primarily based on RFLP and SSR markers described by Crasta et al (1999) and Xu et al (2000). The 566 DG markers used in map construction were generated using *NgoMIV*, a methylation sensitive restriction enzyme that improved the discovery of markers in the sorghum genome targeting regions of high recombination frequency. WinQTL Cartographer V2.5(Wang S., 2011) was used to generate QTL information. Composite interval mapping at walk speed 1cM and regression run to 1000 permutations was initially set at a likelihood ratio (LR) 11.5, corresponding to a conversion LOD (log of odds) threshold of 3. The LR was adjusted to 50 percentile rank of permutation output above which significant LOD scores were used. Significance level was set at 0.05 and Kosambi correction was used. Appearance of corresponding QTL peaks and sizes were used to infer an association at the appropriate threshold. On this mapping output, trace coordinates intersection of the trace hairs provided the exact LOD scores for the significant QTL peaks. Trace hair peaks for the CTD and EWL plots corresponding to the significant LOD peaks were used to evaluate proportion of phenotypic variance explained by a given QTL. QTL width was measured 1 LOD score from the peak and flanking markers within this range noted.

The QTLs were overlaid on the Stg QTL positions previously published (Crasta, 1999; Subudhi, 2000; Xu, 2000) for the same mapping population. These previously mapped Stg loci were created in a study by Xu et al (Xu, 2000) from visual phenotypic rating on 1 to 5 scale. 1 corresponded to total leaf senescence, and 5 represented little leaf senescence at grain maturity. Using similar scoring, other studies have confirmed and discovered additional loci (Harris, 2007; Kassahun, 2010; Sanchez, 2002) and (Crasta, 1999; Subudhi, 2000) for the staygreen trait in other regions of sorghum genome. Corresponding map regions on linkage groups were compared and deductions made.

MapChart V2.2 (Voorrips, 2002) was used to organize the graphical linkage map presentation of the data from WinQTL Cartographer V2.5.

### **3.3 Results and discussions**

#### ***3.3.1 QTL identity for the traits***

A total of 28 QTL spread across 5 chromosomes were identified for temperature depression, leaf wax and flowering time (Table 5 a & b). Most of the alleles associated with positive additive values for wax and TD loci were contributed by the pre-anthesis drought tolerant parent, Tx7000. Alleles for extended flowering time were mainly contributed by the senescence resistant (staygreen) parent, Tx642. In the combined analysis 16 QTL were detected for temperature depression, 9 QTL for cuticular leaf wax and 3 QTL for flowering time. Table 5 and Figure 7 summarize these results.

On chromosome 10, both QTL *TDwl-10-58* (PVE=11.1) for temperature depression and that for leaf wax *WLwl-10-58* (PVE=12.6%) (also found in a second environment, PVE = 13.2) were associated with the same marker *c10B13413* located at 108.2cM position. This suggests they are possibly tightly linked or have pleiotropic interaction.

Of the 3 QTL detected for flowering time, one was on chromosome 8 and two on chromosome 10. In both cases, they overlapped with either QTL for temperature depression or QTL for leaf wax, or both.

On chromosome 8, QTL for temperature depression *TDcx-8-15* at 55.8cM collocated with cuticular leaf wax QTL *WLcx-8-17* at 57.7cM suggesting possible linkage between these two QTL. Flowering time QTL (*FTcx-8-15*) also overlapped that for leaf wax (*WLcx-8-17*, at 57.7cM), and was centered at the same marker position (55.8cM) as that for temperature depression, suggesting pleiotropic interactions of these two QTL. This flowering time QTL explained 11.7% of the phenotypic variation. The QTL for leaf wax and QTL for temperature depression explained 11.5% and 19.6% of the phenotypic variance. It is possible that a group of tightly linked genes at this locus affect these three traits. All the three QTLs were detected in the same environment. Possible linkage between QTL for temperature depression and that for flowering time were also detected on Chromosome 10 in a combined analysis. QTL for temperature depression (*TDwl-10-33*) associated with the marker *c10B4064* at 62.6cM (PVE=19.6) collocated (1.2cM apart) with QTL *FTcx-10-35* (also detected in a second environment) associated with the marker *c10F4552* located at 61.4cM. Two QTL for leaf wax (*WLcx-10-58* and *WLwl-10-58*) associated with the marker *c10B13413* at 108.2cM were detected on chromosome 10 in two field environments. These QTL were closely linked to QTL for temperature depression (*TDwl-10-58*) associated with the same marker and detected in same environment as one of the leaf wax QTL. There is a possible pleiotropic interaction between these QTL. On the same chromosome, a QTL for leaf wax (*WLcx-10-55*) and temperature depression (*TDwl-10-54*) collocated, and their representative markers 5.5cM apart at 99.9cM and 94.5cM, respectively.

On chromosome 6, temperature depression QTL (*TDcs-6-14*) and leaf wax QTL (*WLcs-6-14*) were detected in the same field environment, associated with the maker *c6B8118* located at position 46.8cM. The PVE attributed to the QTL were 13.5 and 12.1,

respectively. This further suggests tight linkage and pleiotropic interaction between the QTL for these two traits.

The average effects of all significant QTL of alleles from each parent are shown of Figure 5, while some of the significant QTL found to be overlapping other QTL or detected across many environments are summarized in Figure 6 (a) and (b). This listing is not exhaustive; it is mean solely to show the pattern of effects they have corresponding to the allelic source parent.

**Table 5:** QTL detected in BTx642/RTx7000 Mapping Population (n=90) in the greenhouse (a) and field locations (b).

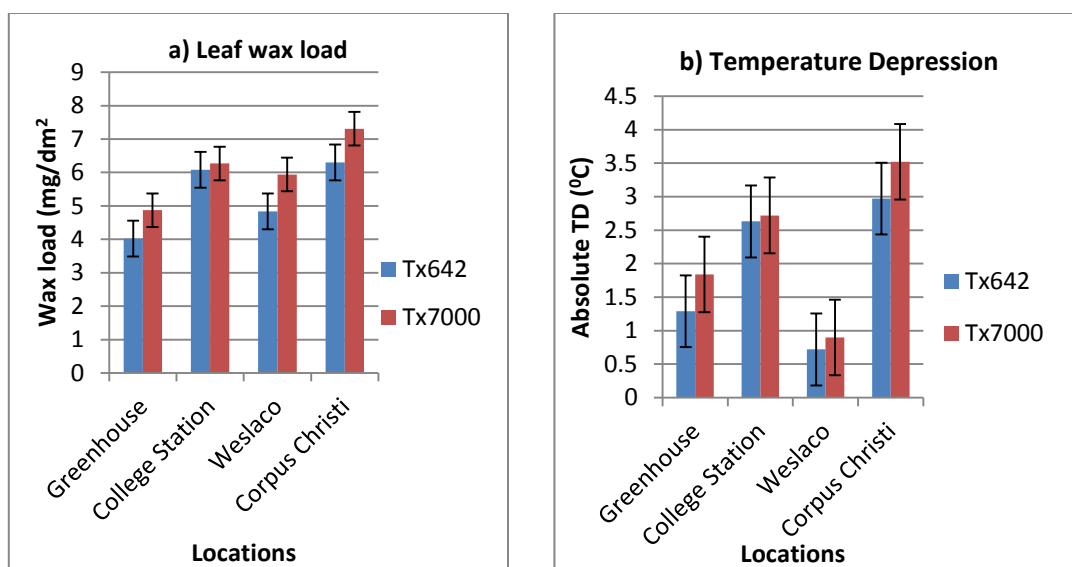
*a) Greenhouse*

QTL	Markers	Position	LOD score	Additive	R <sup>2</sup>	Positive allele	Environment detected
<i>Leaf cuticular wax</i>							
<i>WLgh-3-86</i>	c3F19429	131		0.1222	0.1049	<b>Tx7000</b>	Gh, Wl
<i>Temperature depression</i>							
<i>TDgh-3-74</i>	c3B17049	104.6	2.6504	-0.2204	0.0986	<b>BTx642</b>	Gh
<i>TDgh-8-1</i>	c8B168	4	2.8813	-0.2399	0.1132	<b>BTx642</b>	Gh
<i>TDgh-8-4</i>	c8F787	11	3.3425	-0.243	0.1136	<b>BTx642</b>	Gh
<i>Flowering time (days to flowering)</i>							
<i>FTgh-10-35</i>	c10F4552	61.4	7.7535	8.3466	0.1572	<b>Tx7000</b>	Gh, Cx

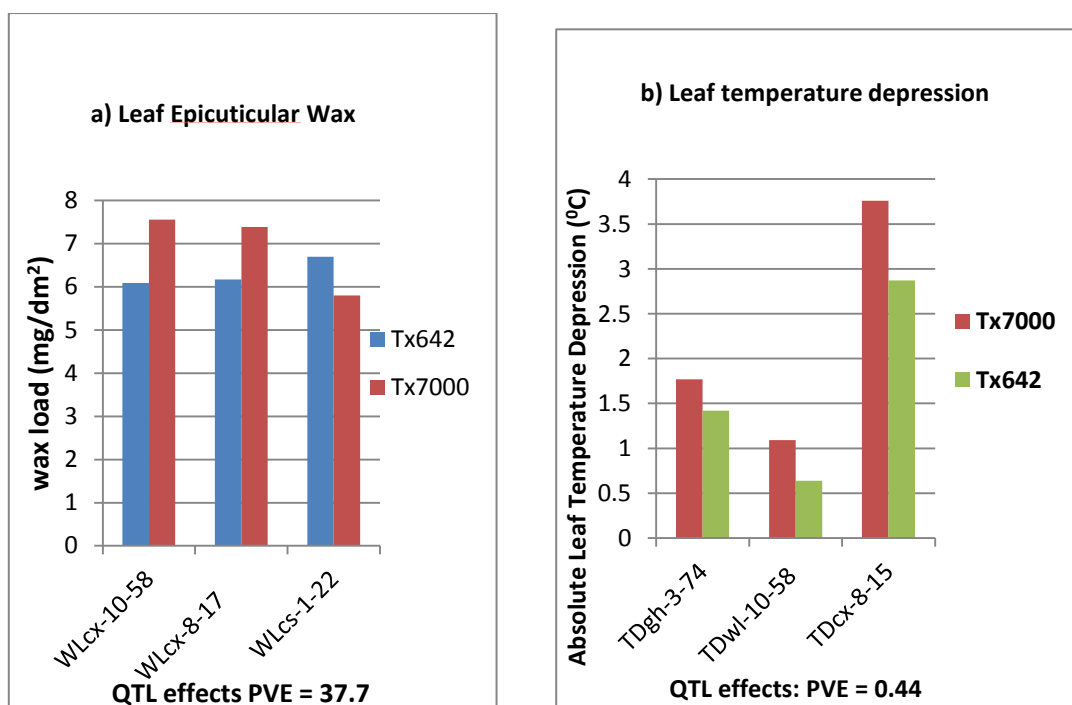
**Table 5:** Continued*b) Field locations*

QTL	Markers	Position	LOD score	Additive	R <sup>2</sup>	Positive allele	Environment detected
Leaf cuticular wax							
<i>WLcs-1-22</i>	c1F5510	38.4	3.2668	0.147	0.1293	<b>Tx7000</b>	Cs
<i>WLcs-6-14</i>	c6B8118	47.2	3.4709	-0.1434	0.1208	<b>BTx642</b>	<b>Cs</b>
<i>WLwl-3-86</i>	c3F19429	131	3.3304	0.1218	0.1097	<b>Tx7000</b>	Wl, Gh,
<i>WLwl-10-58</i>	c10B13413	107	3.4668	0.126	0.1267	<b>Tx7000</b>	Wl, Cx
<i>WL-cx-8-17</i>	c8B9861	57.7	3.1846	0.1219	0.1151	<b>Tx7000</b>	<b>Cx, Wl</b>
<i>WL-cx-10-50</i>	c10B12119	83.9	2.6205	-0.1322	0.0917	<b>BTx642</b>	Cx
<i>WL-cx-10-55</i>	c10B13167	99.9	2.8261	0.1401	0.1037	<b>Tx7000</b>	Cx
<i>WL-cx-10-58</i>	c10B13413	108.2	3.6789	0.1397	0.1323	<b>Tx7000</b>	Cx, <b>Wl</b>
Temperature depression							
<i>TDcs-4-67</i>	c4B15449	124.4	3.086	-0.2987	0.095	<b>BTx642</b>	Cs
<i>TDcs-6-12</i>	c6F7661	41.5	3.156	-0.3039	0.103	<b>BTx642</b>	Cs
<i>TDcs-6-14</i>	c6B8118	47.2	4.0535	-0.3528	0.1353	<b>BTx642</b>	<b>Cs</b>
<i>TDcs-8-8</i>	c8F2049	42.3	4.8263	0.3807	0.1609	<b>Tx7000</b>	Cs
<i>TDwl-10-33</i>	c10B3717	52.6	4.3184	-0.3481	0.1654	<b>BTx642</b>	Wl
<i>TDwl-10-34</i>	c10B4064	60.3	5.5191	-0.3813	0.1967	<b>BTx642</b>	Wl
<i>TDwl-10-42</i>	c10B11157	68.6	3.3116	-0.278	0.1118	<b>BTx642</b>	Wl
<i>TDwl-10-54</i>	c10F12818	98.5	3.5743	0.2964	0.1308	<b>Tx7000</b>	Wl
<i>TDwl-10-58</i>	c10B13413	108.2	4.9324	0.3085	0.1578	<b>Tx7000</b>	<b>Wl, Cx</b>
<i>TDcx-4-44</i>	c4B13239	93.5	5.4034	0.4575	0.1647	<b>Tx7000</b>	Cx
<i>TDcx-6-48</i>	c6F12702	98.8	3.2203	-0.4713	0.1023	<b>BTx642</b>	Cx
<i>TDcx-8-9</i>	c8B7863	45	4.2133	0.454	0.1498	<b>Tx7000</b>	Cx
<i>TDcx-8-15</i>	c8F9646	55.8	6.2923	0.5053	0.1964	<b>Tx7000</b>	Cx
Flowering time (days to flowering)							
<i>FTcx-8-15</i>	c8B9861	55.8	3.0584	5.4434	0.1176	<b>Tx7000</b>	<b>Cx</b>
<i>FTcx-10-35</i>	c10F4552	61.4	3.4715	4.9952	0.0812	<b>Tx7000</b>	Cx, Gh





**Fig. 5:** Mean allelic values for traits associated with significant QTLs detected in each environment. QTL for each trait (TD and Wax) are reported separately.



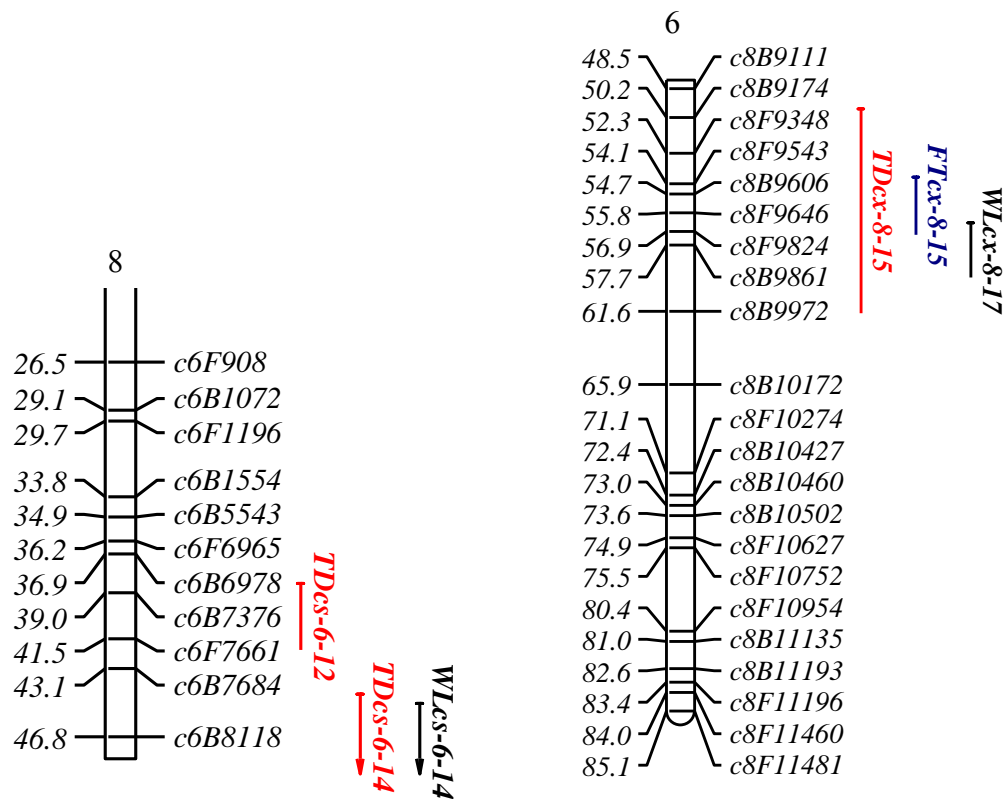
**Fig.6:** Sample mean QTL effects associated with the positive allele. The PVE shown is the sum total for the QTL shown on the horizontal axis

### **3.3.2 Collocation of QTL for staygreen and those for leaf wax, temperature depression and flowering time.**

Although high concentration of QTL for staygreen has been observed on chromosome 2 and 5 in past studies, there were no QTL for the traits investigated under our study, detected on these chromosomes. We are only reporting staygreen QTL which overlap the traits under this study. These were on chromosome 1 and 2. QTL for staygreen 1 (*Stg1-C*) associated with a marker at 105.7cM (with interval 104.0 – 107.5cM) on chromosome 3 detected under partially watered environment, had been earlier been reported by Xu et al (Xu, 2000). In our study we detected QTL for temperature depression (*TDgh-3-74*), under well-watered environment, associated with the marker *c3B17049* at 105.6cM flanked between 101.9 to 106.1cM. Clearly, these two QTL are tightly linked. Among all the stable staygreen QTL, the staygreen 1 has been reported as having second highest phenotypic effect while stay green 2 was the most significant under drought (Crasta, 1999; Sanchez, 2002; Xu, 2000). The temperature depression QTL explained only 8% phenotypic variance.

On chromosome 1, QTL for leaf wax (*WLcs-1-22*) was detected at 38.4cM, explaining 12.9% phenotypic variance. This QTL overlapped that for staygreen 2 (*Stg2-A*). Past studies have identified and mapped the four staygreen QTL, *Stg 1, 2, 3* and *4* at post-anthesis stage. And stable QTL for these loci have been mapped to the same chromosome 1, 2, 3 and 5 in various environments over a number of years (Crasta, 1999; Xu, 2000). Figure 5 shows that Tx 7000 contributed most of the positive alleles conferring high leaf wax means for all the environments. This is in agreement with the averages of the sample significant QTL shown in Figure 6 apart from the *WLcs-1-22* locus. The effects of this QTL violate the overall trend mean effects associated with the positive alleles from the two parents. It shows the positive allele associated with high wax is from Tx642 which is post-maturity staygreen but pre-flowering drought susceptible. The genetic interaction between these loci may likely be responsible for

this deviation. Furthermore, the detection and co-localization of the Stg locus with cuticular leaf wax locus may indicate that the Stg QTL overlap in time with those conferring drought tolerances at anthesis. It appears staygreen trait expression may be triggered earlier than previously believed. The onset of expression of the different staygreen loci may vary with stress level. The Stg2 locus, which has been identified as the most significant of all the staygreen QTL(Xu, 2000), may be expressed much earlier at anthesis under dry conditions. Our results suggest that that genes controlling staygreen trait may be linked with those controlling leaf wax to influence plant responses to cooling at the reproductive stage.



**Fig. 7:** Graphical representation of linkage groups on chromosomes 1, 3, 6, 8 and 10. Only parts of the chromosome are shown. The numbers on top of each center bar represents the chromosome number. Numbers to the left are genetic distances in cM. To the right are the QTL bars where the capital letters represent the trait identity: TD- temperature depression, FT-flowering time, WL- leaf wax, and S- staygreen.

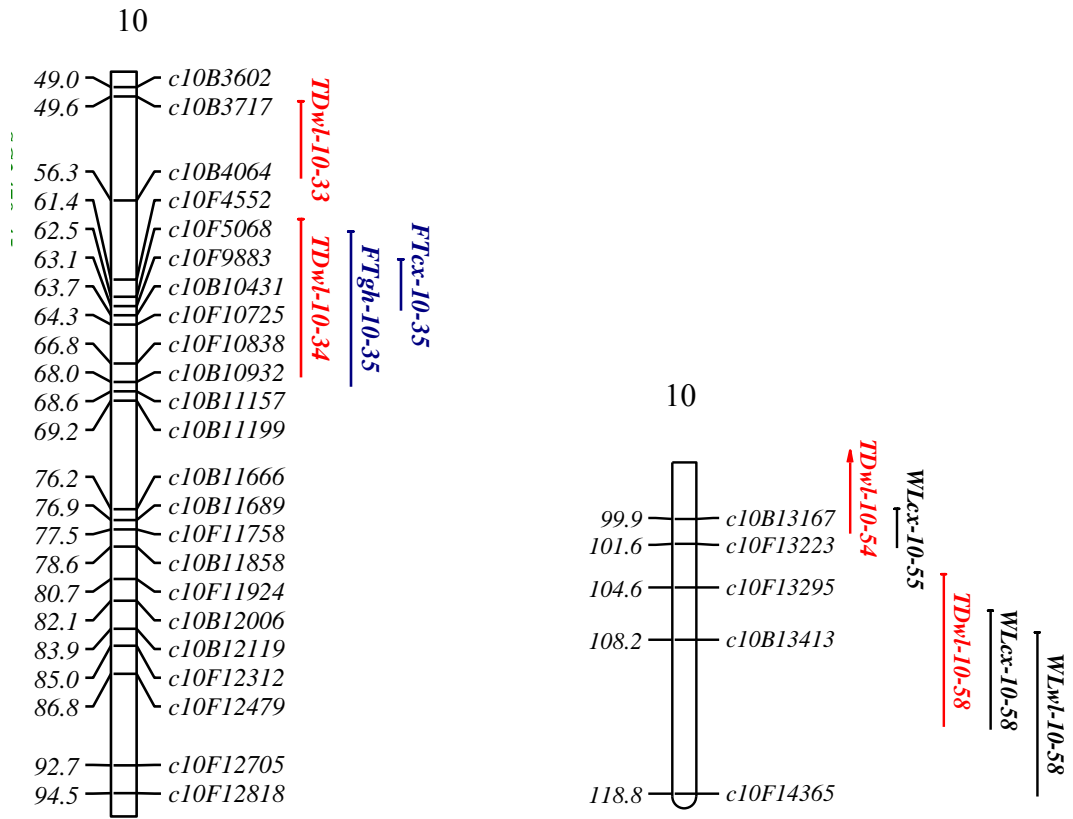


Fig. 7: Continued

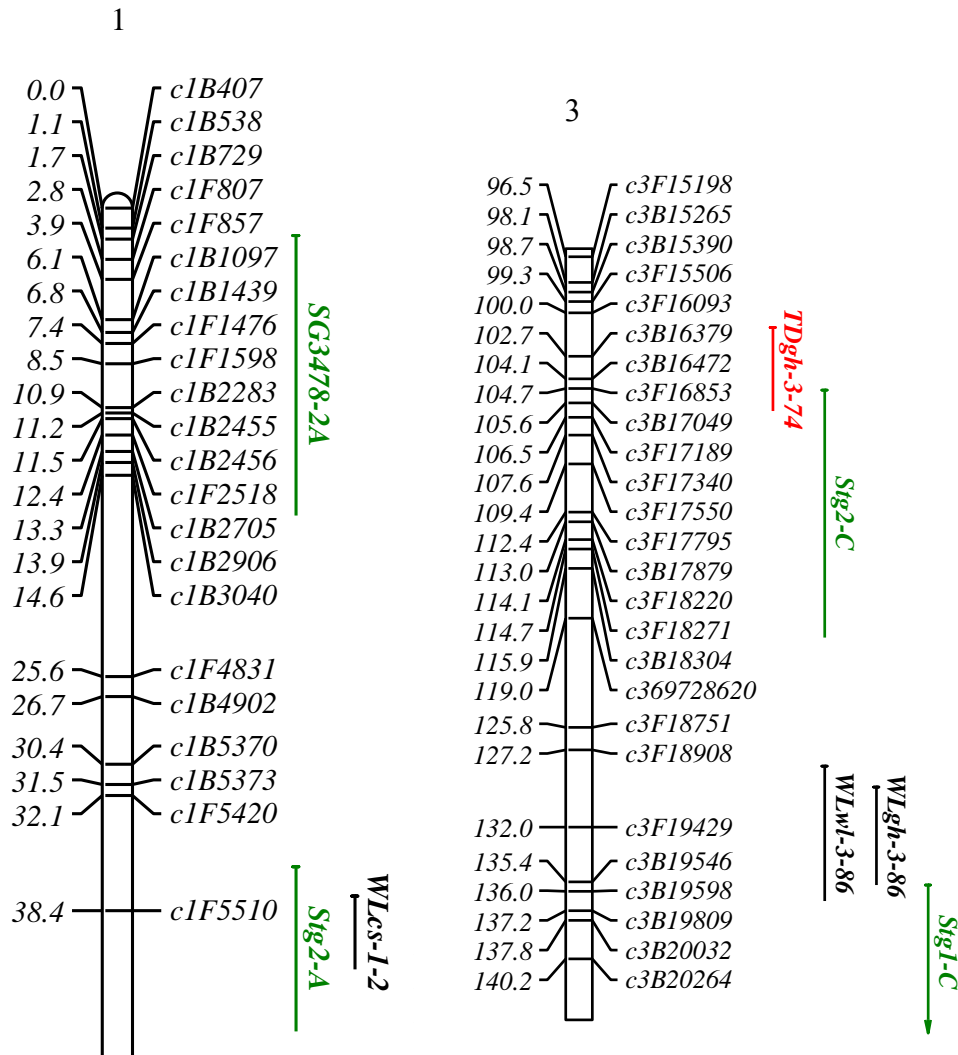


Fig. 7: Continued

### 3.4 Conclusion

This study investigated the genetic overlap between QTL for staygreen, leaf wax and temperature depression. A numbers of traits co-localized to the same QTL. The results indicate a possible strong linkage between QTL for cuticlur leaf wax and canopy temperature depression. The effects of the main overlapping QTL corresponded to the effects expected of the parents contributing the positive alleles. Flowering time wax also captured. The tight linkage between staygreen and wax suggested it is possible

that this Stg2 locus may be important in both pre-anthesis and post-anthesis drought tolerance. We have shown here that there is an important overlap of two QTL that have normally been associated with drought tolerance at reproductive stage. The overlap between QTL for cuticular leaf wax and QTL for staygreen exhibits a departure from the QTL overlaps for other traits with that of cuticular leaf wax. Further study of these major QTL effects on water use efficiency and yield could provide useful information for possible QTL cloning and introgression into breeding germplasm.

It may be useful to determine how QTL for cuticular wax are also expressed at the post-anthesis stage. This may point to the extent of the association between the staygreen and leaf wax traits and provide further impetus to cloning this overlapping QTL for further testing and improvement in drought tolerance.

## CHAPTER IV

### SUMMARY/CONCLUSIONS

Drought has remained limiting factor in sufficient in partitioning of water use for agriculture and other uses. Crop improvement measures aimed at reducing water demand while maintaining optimal productivity have become imperative improvements in drought tolerance strategies. Leaf wax and staygreen traits have been identified as some of the key drought tolerance phenotypes. In this study, we looked at the genotype and environment effects on leaf wax and canopy temperature depression and their variability. We also focused on determining the association between the QTL regulating the staygreen trait in sorghum with improved canopy temperature depression (CTD) as regulated by total and compositional epicuticular wax content in a recombinant inbred sorghum population derived from BTx642 and RTx7000. Phenotypic data were collected in 3 replicated field trials and 1 greenhouse trial. The study has dissected different comparisons of leaf wax and canopy temperature depression as drought tolerance parameters under the watered and dry conditions.

There was a stronger positive correlation between leaf epicuticular wax load (EWL) and canopy temperature depression (CTD) under drought than irrigated field conditions. Plants with higher leaf EWL have cooler canopies showing lower transpirational demand. Generally, staygreen genotypes among the sorghum RILs are able to maintain cooler canopy than the non-stay-green genotypes under drought and hot conditions. Epicuticular leaf wax load was also correspondingly higher in the staygreen genotypes, than non-staygreen. We have related our results and those based on previous studies to infer that high leaf wax may play important role in preventing yield losses in low soil water and high temperature among the genotypes with shorter days to flowering. We have suggested that wax might offer a more stable indicator for selection of drought tolerance under a variety of weather conditions.

These results have been further strengthened by the possible genetic interaction we have demonstrated between staygreen and leaf wax in sorghum. Composite interval mapping identified a total of 28 QTL, fifteen of which had significant overlaps. The overlap between QTL for cuticular leaf wax and QTL for staygreen exhibits a departure from the QTL overlaps for other traits with that of cuticular leaf wax. We report that, just like staygreen and canopy temperature depression, both leaf wax and staygreen also have the potential selection under dry and hot conditions. We have also suggested that under drought stress, the genes for staygreen may be expressed earlier in time (at anthesis) than had been previously believed.



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