REMOTELY ESTIMATING THE INFLUENCE OF EPICUTICULAR WAX ON PLANT HEALTH AND WATER CONTENT USING BLOOM AND BLOOMLESS SORGHUM GENOTYPES

A Thesis

by

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ABSTRACT

Sorghum (*Sorghum bicolor* L. Moench) is an important crop grown for both fodder and food, especially in Africa and Asia. Although it can cope with water shortage, long-term stress affects sorghum growth and productivity. The presence of epicuticular wax (EW) on the vegetative body of sorghum is one effective mechanism for conferring drought resistance. The heavy wax covering is termed "bloom," while bloomless types lack EW layer. The presence of EW has been associated with low abiotic stress tolerance, as each phenotype is associated with the wax composition and not the wax content. However, to monitor the influence of this drought-adaptive trait on plant growth, health and water use efficiency is limited by the unavailability of suitable phenotyping methods. The high resolution hyperspectral imagery consists of more than 200 narrow spectral bands that can monitor plant responses to drought over critical phenological stages throughout the plant life cycle. This study focused on determining the influence of epicuticular wax on plant health and water use efficiency (WUE) using recombinant inbred line population derived by Stg4*M1789. Physiological data was collected from field trails in CS and a greenhouse experiment. The EW data and hyperspectral data was collected at different phenological growth stages at the same time. Our results confirmed that under well-watered conditions the high EW lines had higher WUE compared to low wax lines, but the low wax lines had adapted a short-term resistance to water loss under water deficit conditions. Differences were observed in the reflectance pattern between the lines across the photosynthetic active (400-700nm) region and the near infrared (700-1200nm) region. The lines with consistently high and low wax were measured for their carotenoids content, plant greenness, water content and photochemical reflectance with a marked difference being found in their pattern.

The reflectance index tends to increase in case of carotenoids pigments for low wax lines as the age of plants increase and senescence. Water band and green normalized vegetation index does not seem to be affected much by the presence of epiculticular wax and is considered a more robust estimation of the water and biochemical content. The plant senescence reflectance index increased with a decrease of epicuticular wax during the vegetative stage of plant development and continued into extended plant maturation compared to high wax genotypes indicated canopy stress for low wax lines. The Spectral-Indices related vegetation increases with an increase in EW, which indicates the overall plant health and growth for RILs with higher leaf wax content.

DEDICATION

I dedicate this work to my beloved parents, Mr. Noshad Khan and my Mother Hussan Saba for their unconditional love, support and prayers and my very supporting brothers. They have always encouraged me for higher studies and sent me over here to achieve my dream goal even though no one else in the community supported my education. I am very thankful to all my friends and colleagues who have supported me throughout this journey. Last, but not the least my very supporting and amazing sisters Specially Asma Noshad for always listening to me and guided me whenever needed.

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NOMENCLATURE

Bm	Bloom
bm	Bloomless
CRD	Complete randomized design
CRI	Carotenoids reflectance index
CS	College Station
DAP	Days after pollination
EMS	Ethyl methanesulfonate
EW	Epicuticular wax
GNDVI	Green normalized difference vegetation index
MSR	Modified simple ratio
NDVI	Normalized difference vegetation index
NDWI	Normalized difference water index
NILs	Near isogenic lines
NIR	Near infrared
PAR	Photosynthetic active region
PRI	Photo reflectance index
PSRI	Plant senescence reflectance index
RCBD	Randomized complete block design
RILs	Recombinant inbred lines
SRI	Spectral reflectance indices
WD	Water deficit
WI	Water index
WUE	Water use efficiency
WW	Well-watered

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction and objectives

Changing climate conditions have affected agricultural productivity worldwide, particularly in Africa and Asia (Enete and Amusa, 2010)**.** Per United Nations survey (2011), the population growth rate will increase to 9.1 billion by the year 2050 and will require an increase in crop productivity at the rate of 2.4% per year (Araus and Cairns, 2014). Drought is a major contributing factor that reduces crop productivity and yields. There is an urgent need to adapt drought avoidance mechanisms for the development of resilient crop genotypes. Work has been done to address this issue, but less attention has been given to utilization of physiological attributes to improve crop productivity and water use efficiency. *Sorghum bicolor (L.)* Moench is the world's fifth most important cereal and a source of food for around 750 million people worldwide (Joint, 2011). It can cope with water shortage, yet long-term stress affects sorghum growth and productivity (Assefa et al., 2010). The presence of epicuticular wax (EW) on the vegetative body of sorghum is one effective mechanism executed for drought resistance (Al-Hamdani and Barger, 2003; Blum et al., 1989). The waxy cuticle protects the leaves and reduces evapotranspiration (Ramatoulaye et al., 2016). The heavy wax covering is referred to as "bloom" while bloomless types lack EW layer. The presence of EW has been associated with low abiotic stress tolerance, as each phenotype is associated with the wax composition and not the wax content. The EW has been shown to protect plants from excess photosynthetic light, resulting in reduced evapotranspiration and normalized temperature (Mondal et al., 2015).

A study found that the water loss through transpiration of waxy genotypes was decreased from 10 to 32% compared to low wax genotypes (Chatterton et al., 1975). Similar results were found in rice, with decreases in transpiration as the wax content increases (O'Toole and Cruz, 1983). However, to monitor the influence of this drought adaptive trait on plant growth, health and WUE is limited by the unavailability of suitable phenotyping methods.

In order to increase the breeding efficiency and select the best-performing lines on large scale, we need more efficient and accurate phenotypic methods (Araus and Cairns, 2014). The current methods available for estimation of plant growth and water use efficiency on a large scale are destructive, slow and labor intensive to record a single measurement per unit time (Rebetzke et al., 2013). Remote sensing uses non-destructive imaging (White et al., 2012) to provide an accurate characterization of a large number of plants in a field**.** The high resolution hyperspectral imagery consists of more than 200 narrow spectral bands (Govender et al., 2007) to monitor plant responses to drought over critical phenological stages throughout the plant life cycle. Spectral indices can be derived by transforming the digital counts extracted from vegetation into reflectance values (Bannari et al., 1995). The key physiological traits derived from these indices for measuring plant health over time include plant greenness, chlorophyll content, photosynthetic activity, carotenoids, light use efficiency, vegetation water content and soil moisture. Any association between EW and these spectral indices can be used to estimate the influence of EW on overall plant health and growth under drought stress. Differences observed among these indices correlating to the wax content can help to define wax's role in drought tolerance. Additionally, correlations of wax content with the plant total WUE would provide a better understanding of the influence of EW on individual genotype water use efficiency and overall health.

The *long-term goal* is to improve the selection efficiency for drought tolerance in epicuticular wax producing sorghum genotypes. The hyperspectral imaging indices for vegetation and water stressed canopies would provide a better indication of each genotype proficiency, thus improving their selection efficiency. The *main objective of this project* is to define the influence of epicuticular wax (EW) by considering the relationship between hyperspectral reflectance indices and physiological traits related to plant health and water use. The spectral indices will measure plant health and water use efficiency at critical phenological growth stages, thus identifying the potential influence of the bloom and bloomless phenotypes on contribution to higher yield during stress. *We hypothesized* that combining high throughput phenotyping hyperspectral imaging indices and physiological measurements could provide an accurate estimation of plant health and extended maturation using varying EW phenotypes. Any association found between EW content, plant health and growth in this project will lead us to select this drought adaptive trait and incorporate it into established cultivars for future studies at the genomic level.

The following specific objectives were used to test the hypothesis.

Objective 1. **Define the role of epicuticular wax (EW) in the regulation of plant water use and water-use efficiency.** Under this objective it is hypothesized that the plant's responses to water stress are influence by the presence of epicuticular wax present on it.

Objective 2. **Define the role of epicuticular wax (EW) in the regulation of plant growth, health and extended maturation as measured by spectral indices.** Under this objective it is hypothesized that the spectral indices that best estimate plant growth and productivity is associated with the presence of epicuticular wax on the plant.

1.2 Review of literature relevant to this project

1.2.1 Effect of epicuticular wax (EW) on drought stress

Epicuticular wax (EW) is an efficient and self-healing white powdery layer acting as a protective barrier against different abiotic stresses (Koch et al., 2009; Rebetzke et al., 2013). There is a diverse variation found in epicuticular waxes due to its different chemical compositions on the epidermal layer of plant leaves, which ultimately affects the plant response to environmental stresses. EW reduces cuticular transpiration by reducing the stomatal opening (Premachandra et al., 1992). An increase was observed in the EW load on leaves of tolerant pea genotypes with reduced leaf temperature under water scarcity (Solanki and Sarangi, 2015). Due to water deficit, the leaf temperature fluctuation has provided information about plant water status (Jackson et al., 1981), which has an impact on yield productivity. Different sorghum cultivars were tested for their water use efficiency under well-watered and water deficit conditions. High water use efficiency was recorded for plants under limited water supply, with 1.864 kg grains/m^3 water compared to the plants under well-watered regimes, the lowest one having 1.088 kg grains/m^3 water (Abdel-Motagally, 2010). Removing the EW using chloroform resulted in reduced surface reflectance, which also reduces the abaxial and adaxial surface reflectance (Uddin and Marshall, 1988).

A study was conducted to monitor the effect of epicuticular wax using waxy bloom, sparse bloom and non-waxy bloomless isogenic lines. They found an increase in transpiration rate as EW content decreased. The effective amount of wax on cuticular water loss was suggested to be greater than 0.067 g/cm^2 (Jordan et al., 1983). The 50 days of EW life present on plants will conserve approximately 3000 g per m^2 of water (Richards et al., 1986). Variations in wax content and composition differ among and within different species. A previous study found significant changes in wax composition and amount in soybean genotypes under water limiting conditions (Kim et al., 2007), but a poor correlation was observed between transpiration and epicuticular lipid composition using twenty barley cultivars (Larsson and Svenningsson, 1986). Nonetheless, at present, there is no well-characterized correlation found between wax amount, transpiration and water deficit environment (Goodwin and Jenks, 2005) as it varies species to species.

1.2.2 High resolution hyperspectral derived indices and plant health

Using remote sensing technology for exploring the trait of interest can provide better results without damaging the plants and are comparatively economic (Gutiérrez-Rodríguez et al., 2004). Traditional methods are available for quantification of different pigments and chemicals present in plants, yet these methods do not allow repeated measurements on the same plant throughout development (Sims and Gamon, 2002). Spectral indices can be derived by transforming the spectral information into reflectance values using simple mathematical equations (Bannari et al., 1995). These reflected values are a direct representation of plant physiology including plant morphological factors. The changing reflectance values are responses by physiological traits, including chlorophyll, responding from 400-700 nm while 700 nm to 1200 nm reflect in the near infra-red spectra (Araus et al., 2001). An increase in reflectance from plant surface was observed due to presence of epicuticular wax (Reicosky and Hanover, 1978). Many spectral indices have been used for measuring plant physiological traits including plant greenness, chlorophyll content, photosynthetic activity, carotenoids, light use efficiency, vegetation water content and soil moisture (Kipp et al., 2014). These vegetation indices can be used to accurately monitor canopy health, greenness and plant photosynthetic activity (Gitelson et al., 1996). The common vegetation indices used for measuring plant greenness are normalized vegetation index (NDVI= R_{900}-R_{680}/R_{900}+R_{680}) which consists of near infra-red (R_{900}) and visible red (R_{680}) wavelength bands (Araus et al., 2001; Rouse Jr, 1974). Another very effective vegetation index is called green normalized difference vegetation index calculated as (GNDVI= R_{780}-R_{550}/R_{780}+R_{550}) proposed by (Gitelson et al., 1996).

There are sixty (60) indices available for estimation of chlorophyll, but the best performing indices among all are (R_{780}-R_{710}/R_{780}-R_{680}) proposed by (Datt, 1999) and (MSR= R750-R445/R705-R445) given by (Sims and Gamon, 2002). Other indices providing important information on plant health and development include the plant senescence reflectance index (PSRI= R680-R500/R750). Any increase in the PSRI values represents plant stress and senescence condition (Dutta et al., 2009). Light absorption and utilization during the process of photosynthesis is estimated using the photochemical reflectance index (PRI= R531-R570/R531+R570) proposed by Gamon, et al. (Gamon et al., 1997). A carotenoids reflectance index (CRI= R510/R550) by Gitelson, et al. (Gitelson et al., 2002b) provide the concentration of carotenoids pigment present in plants**.** Monitoring plant water content using remote sensing technology provides better results to explore drought consequences (Claudio et al., 2006). Spectral indices developed for water content measurement from different absorption regions of the electromagnetic spectrum include water band index (WI=R_{900}/R_{970}) (Penuelas et al., 2010) and normalized difference water index (NDWI= R_{857}-R_{1241}/R_{857}+R_{1241}) (Jackson et al., 2004). A positive correlation was found between NDVI and WI, providing a connection between plant health and amount of water. But these indices vary with water content and plant species, making them independent of each other (Claudio et al., 2006).

The proposed research is *significant* in defining the influence of epicuticular wax (EW) on plant health and water use efficiency in sorghum genotypes. The use of remotely acquired hyperspectral imagery to monitor the relative water content and plant health at critical phenological growth stages will identify the potential genotypes with varying EW phenotypes. The genotypes holding plant health the entire growing season and contributing to final grain productivity will lead us to select this drought adaptive trait and incorporate it into established cultivars for future studies at the genomic level.

CHAPTER II

THE INFLUENCE OF EPICUTICULAR WAX (EW) ON THE RATE OF WATER LOSS AND WATER USE EFFICIENCY UNDER WATER DEFICIT AND WELL-WATERED REGIMES

2.1 Introduction

Drought, which is a major constraint for crop productivity, is expected to affect half of the Earth's arable land in the next 50 years (Solomon, 2007), while the world population is also projected to an increase to 9.1 billion by 2050 (Warning and Assessment, 2011). In order to meet the increasing demands of food and fresh water supplies, the crop water use efficiency (i.e., the ratio of whole-plant biomass to cumulative transpiration) should be improved (Balota et al., 2008). Work has been done to address this issue, but less attention has been given to utilization of physiological attributes to improve crop productivity and water use efficiency. Upon water stress conditions, plants modify their physiological and morphological responses to adapt to an environment (Hsiao, 1973). The surfaces of plant leaves consist of a considerable amount of waxes with diverse chemical structure. Despite this variability, two components are of the most interest: One is cutin, which is a polymeric compound consist of a long-chain hydroxyle and epoxy fatty acids (Heredia, 2003).The second is cuticular wax, which consists of straight chain aliphatic and includes secondary metabolites such as triterpenoids, phenylproponoids, and flavonoids. These waxes play significant fundamental and ecological roles in maintaining plant health and productivity (Barthlott et al., 1998). Epicuticular wax (EW) is an efficient and self-healing white powdery layer acting as a protective barrier against different abiotic stresses (Koch et al., 2009; Rebetzke et al., 2013). The EW changes its function and amount in the process of plant development. (Jenks and Ashworth, 1999). It reflects solar radiations and thus reduces radiation heat and water use through transpiration. A study showed reduced cuticular transpiration in sorghum using bloom, bloomless and sparse bloom lines with an increased in EW (Jordan et al., 1984). The same studies were carried out in oats (Bengtson et al., 1978). In rice, the dewaxing of EW from leaves using chloroform resulted in reduced cuticular resistance compared to the stressed plants (O'toole et al., 1979).

A study reported a short-term increase in wax synthesis on leaf surfaces of cotton under water stress conditions (Weete et al., 1978), therefore signifying the potential role of epiculticular wax for drought adaptation mechanisms in plants. From the water loss viewpoint, a positive correlation was found between the high leaf epiculticular wax and reduced transpiration under water deficit conditions. This chapter explores the influence of epiculticular wax on plant water use and water use efficiency using bloom and bloomless wax mutant lines. This will be important in selecting the genotypes adaptable to drought environments, as well as those that performed well under well-watered as well as water deficit conditions.

2.2 Materials and methods

2.2.1 Plant material

A total of one hundred F_5 generation recombinant inbred lines (RILs) developed from a cross of M1789 and Stg4 lines were used. The M1789 is an ethylmethanesulfonate (EMS)-induced bloomless line. Ethylmethanesulfonate is a chemical mutagen with a very high frequency of success in generating the mutagenized population of different plant species (Xin et al., 2008). Stg4 is a bloom line. The stg4, a staygreen character that can be used for developing a drought resistance cultivar, is the female in this single one-way cross.

There were no significant differences found in the segregation patterns of the F_2 reciprocal crosses. Another fifty BC_2F_6 bloom and bloomless near isogenic lines (NILs) derived from ethylmethanesulfonate (EMS) mutagenized Tx623 (Xin et al., 2008), a heavily bloom inbred line. M401 is generally a low-wax load (WL) genotype and the male parent. Tx623 was the recurrent parent. Each line is medium height, or short or slightly taller than Tx623.

2.2.2 Growing environment

A greenhouse experiment was conducted using recombinant inbred lines (RILs) near isogenic lines (NILs) and their parents at the Borlaug Institute (Texas A&M University, College Station, TX) to measure WUE and plant water relation to sorghum. The planting dates were in early January. Measurements were taken throughout the developmental stages from January to March and were harvested in April. Data loggers by ‘EL-USB-2-LCD’ were used to monitor the relative humidity and air temperature for each replication and treatment. The bloom, bloomless and their parental lines were sown in a completely randomized design (CRD) with two replications per treatment. Two sets, ‘early planting’ beginning of January and ‘late planting,’ from late February were planted having two replications each per treatment. Each treatment contained pots with no plants as a standard to measure evaporation from the soil surface. Each set was planted with an interval of three weeks to facilitate measurements for water use efficiency (WUE) and transpiration efficiency.

2.2.3 Physiological measurements

The varying epiculticular wax bloom and bloomless phenotypic lines were tested for their water-use efficiency. The bloom and bloomless genotypes were treated with two water treatments. Well-watered treatment with approximately 2000ml of water and water deficit with 700 ml of water applied. The pots sized 1200 CC were filled with Metromix 366 and Metro mix 900 fine mixed soil. Weed control fabric was used to cover the bottom of each pot to avoid the excessive water loss. The first set was planted on January 18, 2016 with 5 seeds per pot and after germination pots were thinned to 2 plants per pot at 15DAP. The fertilizers consist of macro nutrients (20-10-20) with three primary elements including nitrogen, phosphorus in oxide form, and potassium. The micronutrients (20-20-20) contained basic micro elements including boron, zinc and copper, and were provided 13d after planting. Water treatment remained the same two weeks after germination. Water deficit treatment was executed 14d after germination, which continued with weighing and watering at a 2d interval. An electronic balance was used for weighing purposes with an increment of 5g. Measurements were taken at specific times between 7 pm to 10 pm under the reduced sun light and transpiration. Daily transpiration from each line was recorded by taking the difference between initial and final pot mass. The water loss was summed up to calculate the daily transpiration from plants. For the water use efficiency, total biomass per plant was obtained after 60d of germination at the grain filling stage. Due to unfavorable greenhouse conditions, the harvesting was done at 60d by taking the shoots at soil level along with the grains section. The collected biomass was oven dried at 60 °C and weighed. Using the water use efficiency index, the dried biomass was divided by cumulative transpiration (evapotranspiration) from planting to harvest. A correlation analysis was carried out between epiculticular wax and water use by bloom lines, with heavy wax and bloomless lines with comparatively low wax.

2.2.4 Epicuticular wax quantification

The EW was collected from each RIL and NIL line at 50% anthesis stage. This was handled carefully, without touching the leaf surface, to avoid brushing of the EW layer. A total of five flag leaf disks of 7.5mm of diameter were collected into clean vials using a rabbit tool. Wax extraction procedure was performed at the AgriGenomics Laboratory at Texas A & M University. The extraction of wax was done using the colorimetric method described by Ebercon, et al. (Ebercon et al., 1977). The EW from leaf disks was extracted using chloroform solution by dipping the leaf disks for 20 seconds. This time period for wax extraction was previously described by Mayeux Jr and Jordan (Mayeux Jr and Jordan, 1984). The extracted wax was then transferred into a clean 2.0ml GC vial (VWR auto sampler vail, Radnor, PA) using a glass pipette. The solution was put into a fume hood until the chloroform ($CHCl_3$) solution completely evaporated from the EW. When the EW was completely dried, potassium dichromate ($K_2Cr_2O_7$) was used to produce the desired color change. This procedure is based on producing the desired color change due to an oxidation reaction between an acid and a base.

The reagent was prepared by combining 20g powdered $K_2Cr_2O_7$ and 40ml deionized water. The resultant slurry was than heated with 1000 ml H_2SO_4 to get a clear solution. After getting the desired color solution, 0.5ml of the reaction mixture was added into each dried wax-containing vails and thoroughly mixed on vortex mixer for 2s. The samples were heated for 30min in a boiling bath at 100^0C. The sample solutions were left to cool for an hour and 1ml of deionized H_20 was added to each sample. Due to a reaction between acid and water, the solution color changed and heat was produced. A spectrophotometer (PHERAstar plus, BMG LABTECH, Offenburg, Germany) was used to take absorbance values for EW load. Samples were loaded into a clean and flat bottom microplate (Greiner Bio-One, Monroe, NC, USA) containing 96 wells. The optical density was kept at 590nm and the obtained wax values were converted into mg/dm^2 using the Beer-Lambert formula proposed by Ebercon, et al. (Ebercon et al., 1977). A standard curve was derived using the sorghum and carnauba wax as both have a positive correlation with an R^2 value of 0.96.

2.2.5 Statistical analysis

The statistical analysis was performed using JMP Pro 11.2.1 (JMP version 11, SAS Institute Inc., Cary, NC) software. A simple correlation was done to check the linear relationship and the regression procedure was used between leaf epiculticular wax and water use efficiency for varying EW genotypes.

2.3 Results

2.3.1 Epicuticular wax load of bloom and bloomless lines

Analysis of wax load was carried out using recombinant inbred lines and near isogenic lines, noting the differences among their EW. The bloom RILs and bloomless RILs showed a significant difference between wax content (Fig 2.3.). A student t-test was performed to see any significant difference between wax content of RILs with bloom and bloomless phenotypes. Significant differences were found with the probability value of 0.0001. The mean for Bm and bm phenotypic wax content was 3.89 mg/dm^2 and 1.50 mg/dm^2, respectively. The Bm and bm had minimum 0.580 mg/dm^2 and 0.226 mg/dm^2 and maximum 10.709 mg/dm^2 and 3.288 mg/dm^2, respectively. A weak but significant correlation with r of 0.23 was found between Bm and bm RILs at $p<0.05$ significance level. The NILs were also tested based on varying EW content. A student t-test was performed between Bm and bm epiculticular wax content with a probability value 0.163, with no significant difference in the EW content (Fig. 2.4).

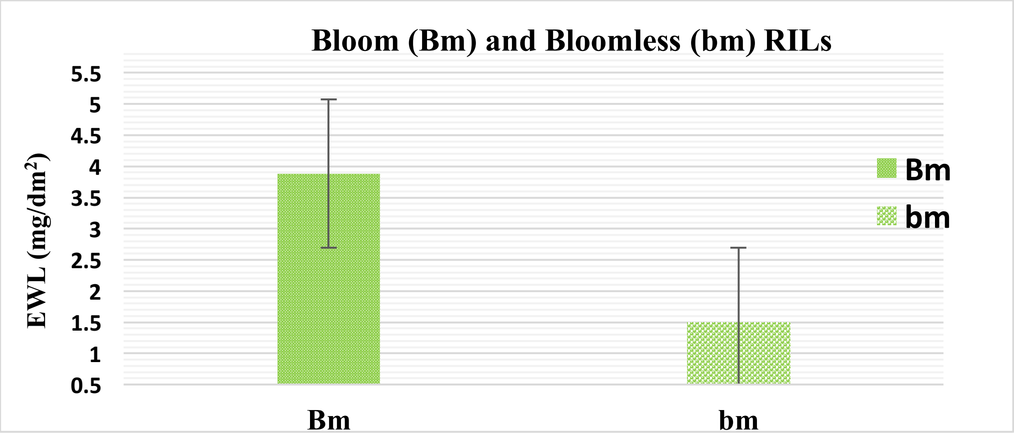

Fig. 2.1. The mean bloom and bloomless epiculticular wax content in (mg/dm$^{2)}$). The Bloom (Bm) recombinant inbred lines (RILs) from a cross of M1789 and Stg4 had significantly higher wax content compared to the bloomless (bm) counterpart.

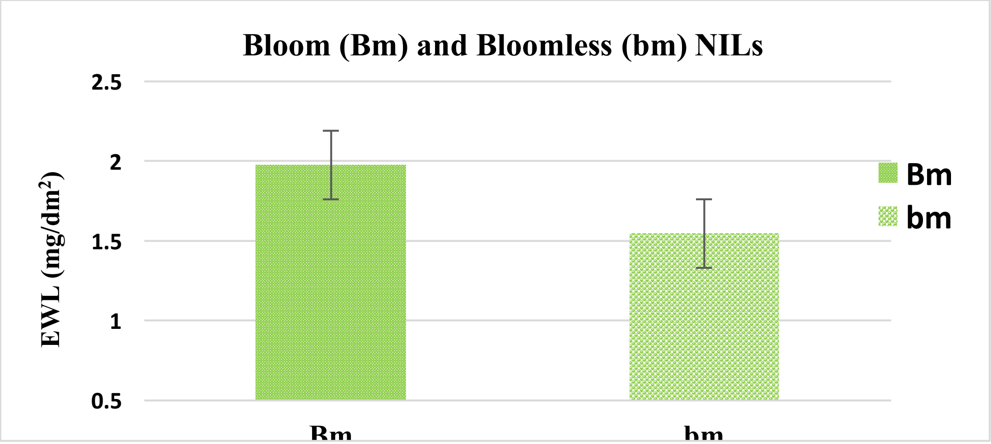

Fig.2.2. The mean bloom and bloomless epiculticular wax content in (mg/dm$^{2)}$). The Bloom (Bm) near Isogenic lines (NILs) from a cross of Tx623 and M401 has slightly higher wax content compared to its bloomless (bm) counterpart.

2.3.2 Water use efficiency under well-watered and water deficit treatment

The mean water loss between Bm and bm recombinant inbred lines had a significant trend throughout their developmental stages. The Bm lines with more EW represented as XS1 transpired less water compared to the lines with low wax represented as XS2 under water deficit treatment (Fig.2.5.). Under well-watered treatment the water loss trend varies across different developmental stages between Bm and bm lines (Fig.2.6.). The bloom NILs and bloomless NIL had no significant difference in the mean water loss under well-watered and water deficit treatments (Fig.2.7 and Fig.2.9).

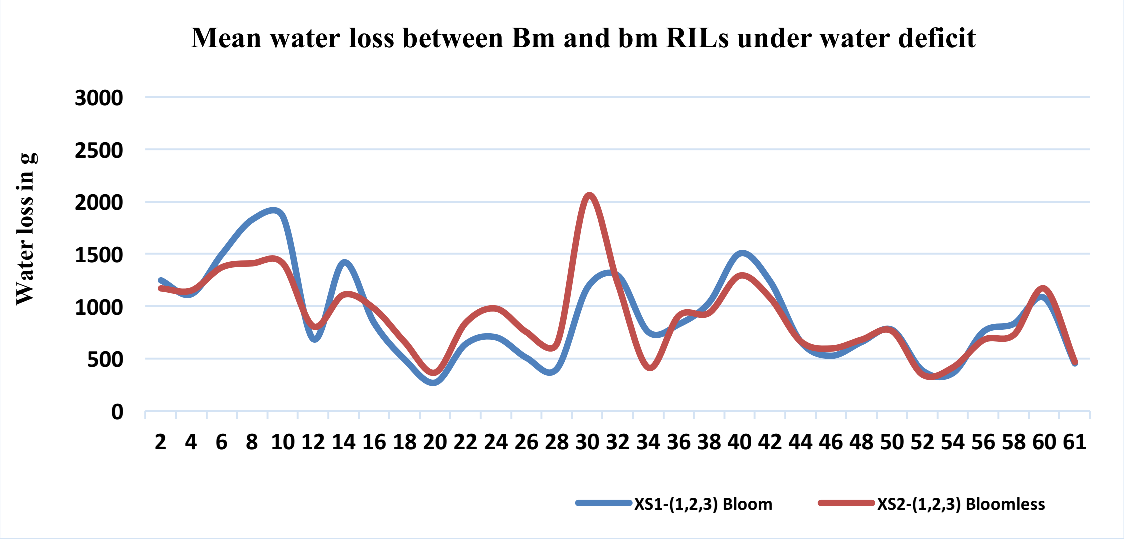

Fig. 2.3. The mean water loss between Bloom and bloomless F4 RILs from the Stg4 * M1789. Data points are the differences of 2 days' interval between water loss under the water deficit treatment

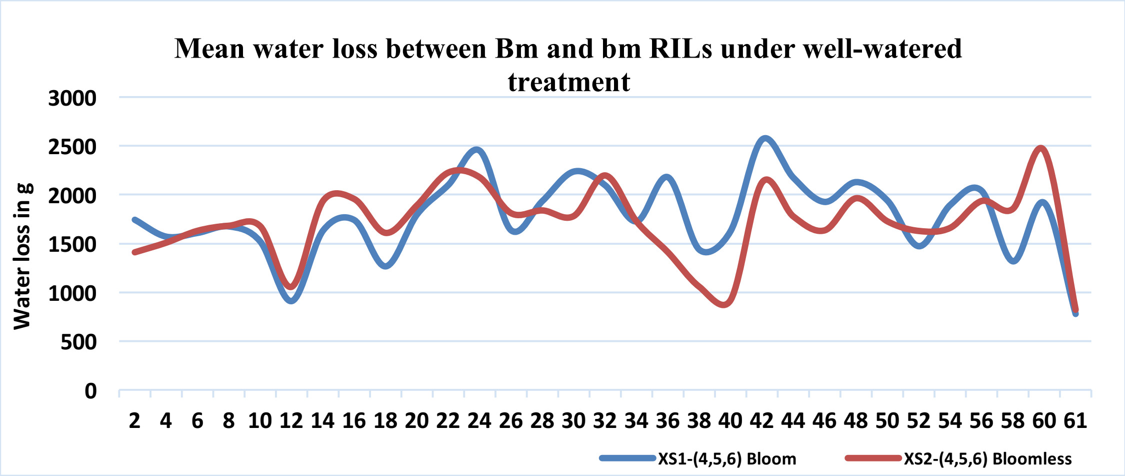

Fig. 2.4. The mean water loss between bloom and bloomless F4 sergeants from the Stg4*M1789. Data points are the differences of 2 days' interval between water loss under well-water treatment

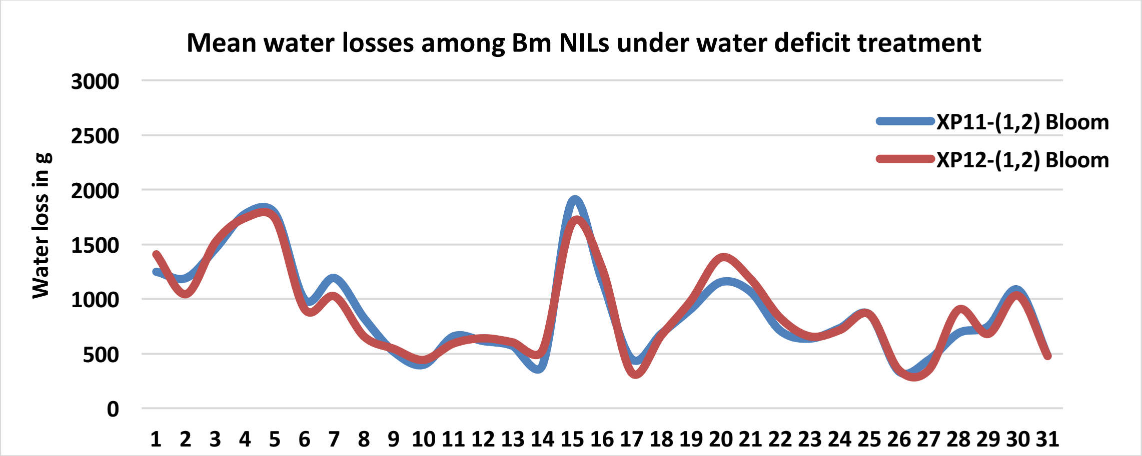

Fig. 2.5. The mean water loss among bloom (Bm) BC2-F5 near Isogenic lines (NILs) From M401 X 623 under water deficit treatment. The XP11 and XP12 are the derivatives with bloom phenotype.

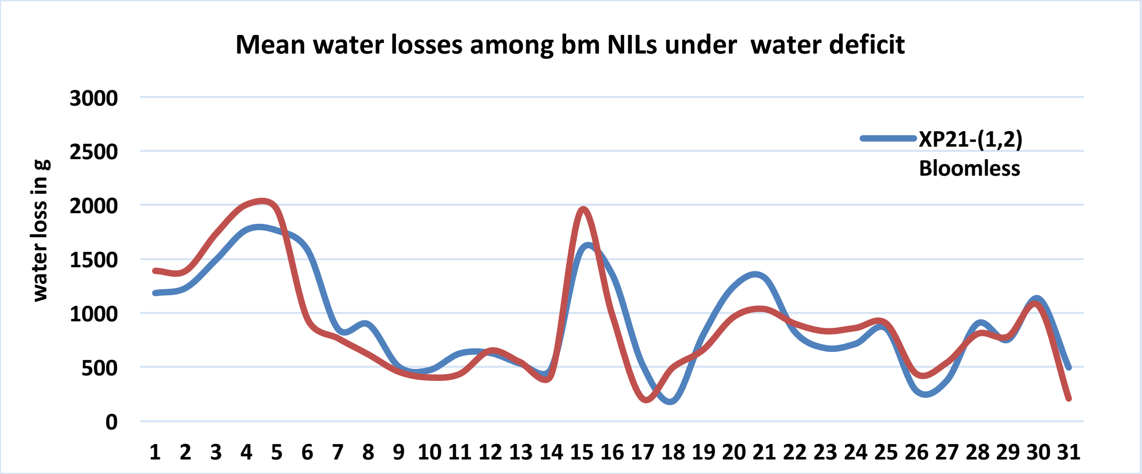

Fig. 2.6. The mean water loss among bloomless (bm) BC2-F5 near Isogenic lines (NILs) from M401 X 623 under water deficit treatment. The XP21 and XP22 are the derivatives with bloomless phenotype.

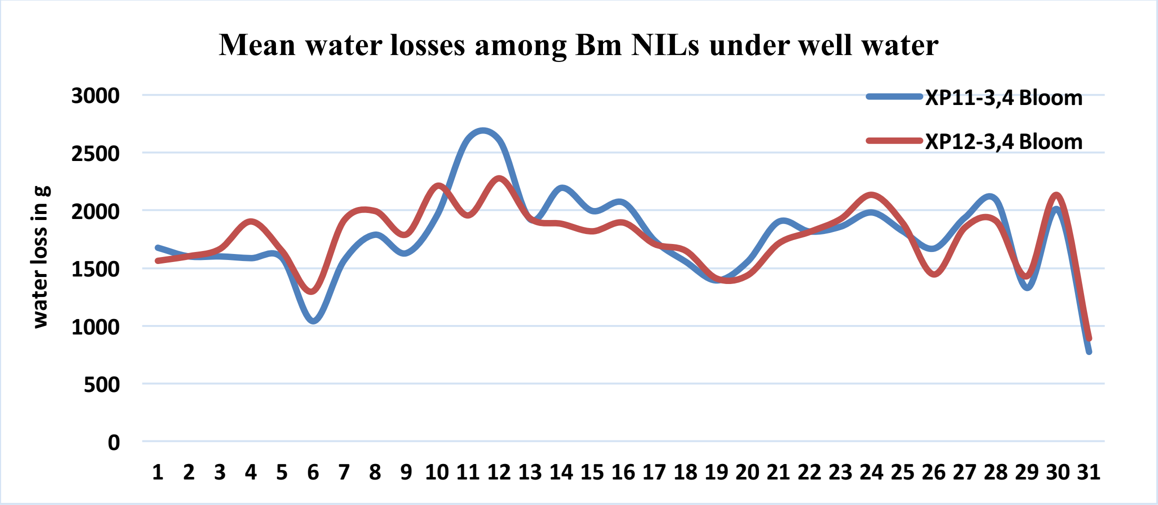

Fig. 2.7. The mean water loss among bloom (Bm) BC2-F5 near Isogenic lines (NILs) from M401 X 623 under well-water treatment. The XP11 and XP12 are the derivatives with bloom phenotype.

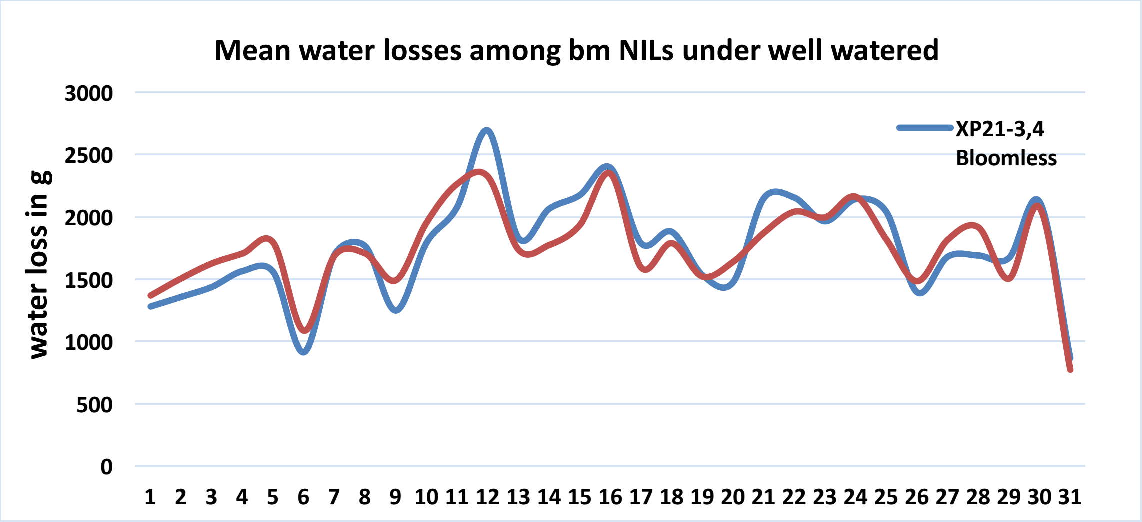

Fig. 2.8. The mean water loss among bloomless (bm) BC2-F5 near Isogenic lines (NILs) from M401 X Tx623 under well-watered treatment. The XP21 and XP22 are the derivatives with bloomless phenotype.

2.3.3 Integrated water use efficiency

The ratio of plant water used during plant metabolism and the water loss by the plant through transpiration was calculated. The total biomass was measured per phenotype for RILs and NILs under well-watered and water deficit treatment. The WUE of F_4 bloom recombinant lines with high wax represented as XSI (Bm) was significantly higher compared to the bloomless recombinant lines represented as XS2 (bm) under well-watered treatment. No significant differences were found among NIL under well-watered treatment, with varying EW content represented as XP11 and XP21 for bloom and bloomless phenotypes, respectively (Fig.2.11). Surprisingly, under water deficit conditions the WUE of bloom recombinant lines, which are represented as XS1 and bloomless as XS2, were not significantly different. The NILs of bloom and bloomless (represented as XP12 and XP22, respectively) with no differences in their wax content were also not significantly different under water deficit treatment (Fig.2.12).

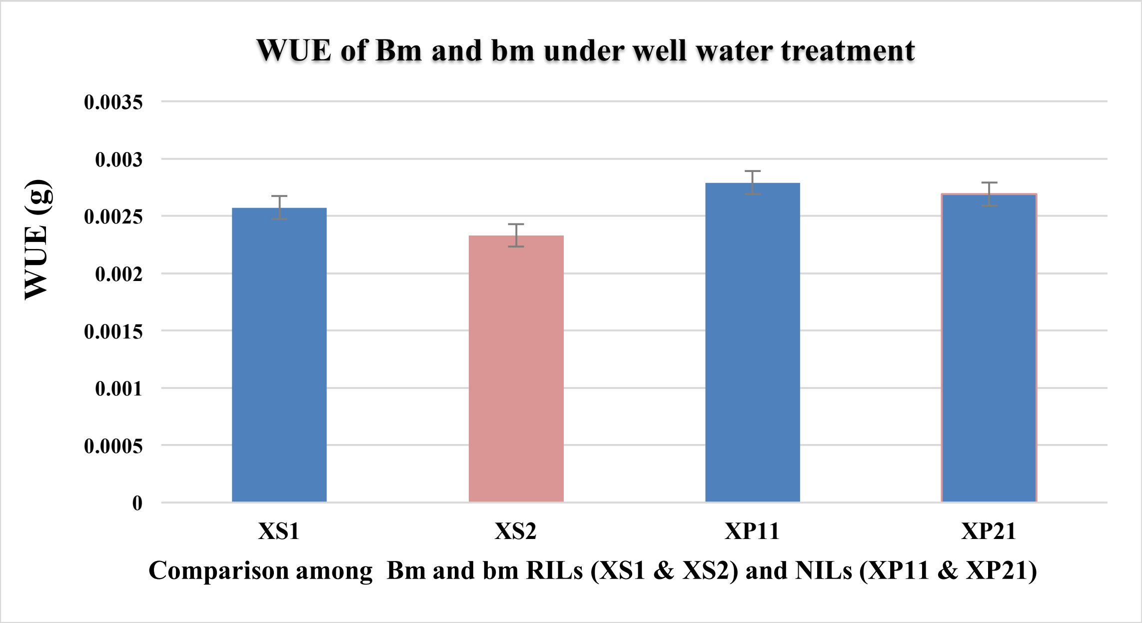

Fig. 2.9. The water use efficiency (WUE) of bloom (Bm) and bloomless (bm) recombinant inbred lines (RILs) and near Isogenic lines (NILs) under well-watered treatment. The F4 Bm with a higher wax load from the Stg4 X M1789 represented as XS1 (Bm) had a significantly higher WUE than F4 bm represented as XS2. No significant difference was found between near isogenic Bm and bm lines represented as XP11 and XP21 respectively.

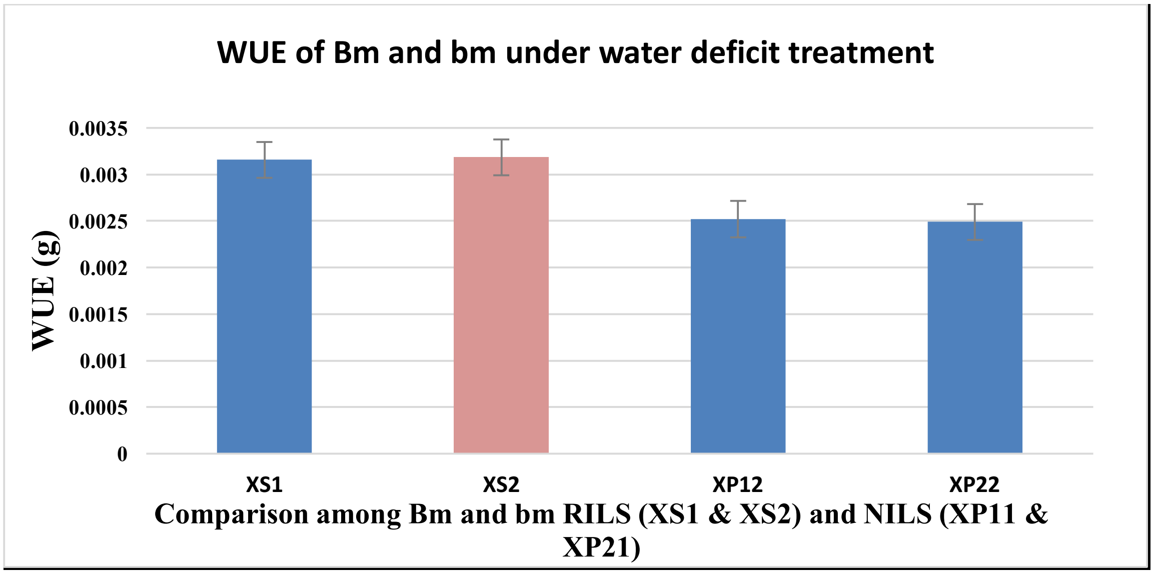

Fig. 2.10. The water use efficiency (WUE) of bloom (Bm) and bloomless (bm) recombinant inbred lines (RILs) and near Isogenic lines (NILs) under water deficit treatment. The F_4 Bm with a slightly higher wax load from the Stg4 X M1789, represented as XS1 (Bm) had no significant difference for WUE than F_4 bm represented as XS2. No significant difference was found between near isogenic Bm and bm lines represented as XP11 and XP21 respectively.

2.4 Discussions

2.4.1 Distribution of leaf epiculticular wax of bloom and bloomless genotypes

The results of this study showed that epiculticular wax of bloom recombinant inbred lines were significantly higher than the bloomless phenotypes at the significant level of 0.0001 (Fig.2.1). The number of leaf disks and procedure of wax extraction was similar for both phenotypes at 50% anthesis stage. Epicuticular wax of bloom lines were markedly higher compared to bloomless lines. The differences in epiculticular wax in plants is affected by both genetic and environmental factors (Whitecross and Armstrong, 1972). The epiculticular wax deposited on the lower leaf surfaces of bloom lines while the wax on the bloomless sorghum lines was thinner than that of the normal lines (Jordan et al., 1984). The epiculticular wax from bloom and bloomless NILs were slightly different at the significance level of 0.05. The presence of EW has been associated with low abiotic stress tolerance as each phenotype is associated with the wax composition and not the wax content. It has been shown to protect plants from excess photosynthetic light, resulting in reduced evapotranspiration and normalized temperature (Mondal et al., 2015). Previous studies done by (Richards, 1983) showed the effect of wax on WUE of wheat lines under dryland field conditions. In this study the wax load for bloom and bloomless lines were as low as 0.580 mg/dm^2 and 0.226 mg/dm^2 and as high as 10.709 mg/dm^2 and 3.288 mg/dm^2, respectively. The RILs wax load for bloom such as XP11 and bloomless such as XP22 were not consistent as some lines had a 1:1 ratio for wax load while others had varying ratios like 1:3 and 1:2 for bloom XS1 and bloomless XS2. The variation among bloom and bloomless RIL were most likely due to the genetic makeup of the different lines.

2.4.2 Water use efficiency

Water deficit conditions, or drought, is a major crop productivity constraint and can disturb the genetic growth potential of the organism. The water use efficiency enhances by increasing the total biomass produced with the same amount of water use, with the decreased amount of water, or it can be a combination of both (Blum, 2005). In this study the water use efficiency of RILs and NILs were tested under water deficit and well-watered treatments. The WUE of F_4 bloom recombinant lines, represented as XSI (Bm), with a higher wax content was significantly increased compared to the bloomless recombinant lines, represented as XS2 (bm), with low wax content under well-watered treatment. As found from the previous studies, the epicuticular wax helped to prevent the excessive water loss and ultra-violet radiations (Uddin and Marshall, 1988). No significant difference in the wax content was found among the bloom and bloomless NILs; it resulted in no significant difference in the their WUE as well. As previous studies reported, a positive correlation between biomass and TE compared to water transpired and TE (Xin et al., 2009). The dewaxing of rice (*Oryza Sativa*) leaves using chloroform resulted in a decrease of cuticular resistance (O'toole et al., 1979). Surprisingly, the cuticular resistance of bm lines increased under water deficit treatment. Under drought or water deficit conditions the waxy bloom and non-waxy bloomless did not show a significant difference, which indicated short term adaptation of non-waxy lines by increasing the synthesis of lipids in their leaf tissues to cope with drought (as also reported by (Weete et al., 1978). The findings from our project and previous studies strongly support the idea that under water stress the epiculticular wax is induced more by plants, thus improving their ability to cope with water deficit conditions by decreasing evaporation and maintaining constant water use efficiency. (Jordan et al., 1984; Thomson, 2007).

2.5 Conclusions

For this objective, the water use efficiency of varying epiculticular wax lines was measured. These results suggest that the bloomless has equal potential to accumulate biomass under water stress conditions compared to its bloom counterpart under our experimental conditions. Water use efficiency of bloom is significantly higher under well-water conditions, where the presence of wax explains the resistance of sorghum to water loss. Under water-stressed conditions, the crops prevent the excessive water loss and the EW increases as described by Jordan, et al. (Jordan et al., 1984; Premachandra et al., 1992). The accumulation of EW under water deficit conditions reduces leaf water potential, which results in increased photosynthetic activity of plants.

CHAPTER III

ROLE OF EPICUTICULAR WAX IN THE REGULATION OF PLANT GROWTH AND HEALTH AS MEASURED BY SPECTRAL INDICES

3.1 Introduction

The increasing demand for food and fresh water supplies requires broad breeding and agronomic efforts to meet growing population requirements (Pingali, 2012). Primary abiotic factors affect plants growth and its productivity include heat and drought stress. Changing climate conditions exposes plants to these primary environmental stresses. Plants adapt to stress adaptive strategies by modifying their morphological and physiological trait responses. These traits include the presence of epiculticular wax by reducing the radiation load on plant leaf surface, pigment composition, leaf angle and rolling to increased transpiration efficiency and other stomatal responses. Epicuticular wax covers plant aerial organs to protect them from various biotic and abiotic stresses. Traditional methods are available for quantification of different pigments and chemicals present in plants, yet these methods do not allow repeated measurements on the same plant throughout development (Sims and Gamon, 2002). Remote sensing is an indirect technology used to explore the trait of interest and to convert the spectral information into a readily interpretable form (Bannari et al., 1995). Spectral indices can be derived by transforming the spectral information into reflectance values using simple mathematical equations (Bannari et al., 1995). These reflected values are a direct representation of plant physiology. The changing reflectance values are responses by physiological traits, including chlorophyll, from 400-700 nm, whiles 700 nm to 1200 nm reflect in the near infra-red spectra (Araus et al., 2001). An increase in reflectance from plant surface has been observed due to presence of epicuticular wax (Reicosky and Hanover, 1978). Many spectral indices have been used for measuring plant physiological traits, including plant greenness, chlorophyll content, photosynthetic activity, carotenoids, light use efficiency, vegetation water content and soil moisture (Kipp et al., 2014). This research studied the possible effect of EW on the reflectance of spectral indices related to plant health and growth using low and high waxy lines. These vegetation indices can be used to accurately monitor canopy health, greenness and plant photosynthetic activity (Gitelson et al., 1996).

3.2 Materials and methods

3.2.1 Plant material

In this study a set of 120 F_6 recombinant inbred line (RILs) of sorghum were used. These lines were divided into 60 bloom and 60 bloomless groups based on their visual phenotypes. It was developed from a cross of M1789 and Stg4 lines. The M1789 is an ethylmethanesulfonate (EMS) induced bloomless line. Stg4 is a bloom line. Another fifty BC_2F6 bloom and bloomless near isogenic lines (NILs) were derived from ethylmethanesulfonate (EMS) mutagenized Tx623 (Xin et al., 2008) bloom inbred line and M401, which is a low-wax load male (WL) genotype. The recombinant inbred lines were divided into 12 bloom and 9 bloomless groups.

3.2.2 Growing environment

The RILs and NILs, alongside their parents, were grown in field during the early summer season (April 07, 2016). The experimental location to characterize the EW was the Texas A&M Agrilife field station in College Station. All plots were in a randomized complete block design (RCBD), with each row representing one RIL. Spacing between rows was 38 inches, and each row was 16 inches long. Complete seed emergence occurred 7 days after planting and reached booting, vegetative, grain filling and maturity stages till 2nd week of August 2016. Harvesting was done at the maturity stage.

3.2.3 Physiological measurements

Physiological measurements include leaf EW collection across different phenological growth stages using feek's scale, SRI measurements and yield data. All measurements were taken at specific time between 12 pm to 3 pm.

3.2.3.1 Airborne data collection using hyperspectral imagery

Aerial hyperspectral imagery was collected under clear sky using Cessna 206 aircraft (USDA-ARS). It consists of VNIR E-series imaging spectrometer and a GPS/inertia navigation system (Headwall Photonics Inc., Fitchburg, MA). The hyperspectral camera gathered the information from the electromagnetic spectrum and measured the emitted radiations from the plant surface. The spectral reflectance of canopy was measured from 392 nm to 1009 nm with 5 nm interval. Hyperspectral imagery consists of 120 bands and a special resolution of 0.25 nm (m) at 4500 feet (ft.)

of altitude. For calibration purpose tarps values were calculated at 8%, 16%, 32% and 48% reflectance. Measurements were taken per plot at vegetative, flowering, grain filling and maturity stages. The time to take measurements was kept constant from 11am to 3pm to avoid any differences due to the solar inclination. The lines were grouped into 3 plots per group based on similar phenotype. The spectral information from each group was extracted using ENVI software. A linear regression model for the extracted counts for each waveband and reflectance values were recorded using a high resolution spectro-radiometer, the ASD handheld 2 field spec spectro-radiometer (Analytical Spectral Devices, Boulder, CO). A linear regression was created for the extracted digital counts for each waveband and reflectance values recorded by ASD instrument for each tarp. Reflectance values obtained from the regression equation provided light reflectance for each group. Spectral indices, which are a mathematical representation of any differences in the reflectance at given wavelengths, were calculated. The indices listed in Table 1 were computed to monitor plant health and growth over different phenological growth stages.

Table 1. List of spectral indices calculated in this study

Index	Equation	Reference
Carotenoid reflectance index 1 (CRI)	**$(1/R_{510}-1/R_{550})$**	**(Gitelson et al., 2002a)**
Photochemical reflectance Index (PRI)	**$(R_{531}-R_{570}) / (R_{531}+R_{570})$**	**(Gamon et al., 1997)**
Plant Senescence reflectance index (PSRI)	**$(R_{680}-R_{500})/(R_{750})$**	**(Peñuelas et al., 1994)**
Green Normalized Difference Vegetation Index (G-NDVI)	**$(R_{750}-R_{550})/(R_{750}+R_{550})$**	**(Gitelson et al., 1996)**
Water Band Index (WBI)	**(R_{900}/R_{970})**	**(Penuelas et al., 2010)**

3.2.4 Wax sampling

Wax leaf samples were collected at vegetative, flowering, grain filling and maturity stages using a disc punch tool with a 7.5mm of diameter. A total of five flag leaf punches were collected carefully into clean vials without touching the leaf surfaces to avoid brushing the EW layer. The collected leaf disks were kept in a laminar flow hood to air dry and then put at normal room temperature.

Fig.3.1. Collection of leaf epiculticular wax punches

3.2.5 Wax extraction and quantification

The wax extraction procedure was performed at the Agri-Genomics Laboratory at Texas A&M University. The extraction of wax was done using the colorimetric method described by (Ebercon et al., 1977). Leaf wax was extracted by submerging the leaf disks into a chloroform solution for 20 seconds. The time period for complete wax extraction from plant leaves was previously described by Mayeux Jr and Jordan (Mayeux Jr and Jordan, 1984). The extracted wax solution was transferred into small GC vials using a glass pipette. The solution was then put into a fume hood until the chloroform ($CHCl_3$) solution completely evaporated from the EW. When the EW was completely dried, potassium dichromate ($K_2Cr_2O_7$) was used to produce desired color change. This procedure is based on producing the desired color change due to an oxidation reaction between the acid and the base. After getting the desired color solution, 0.5ml of the reaction mixture was added into each dried wax-containing vial and thoroughly mixed on vortex mixer for 2s. The samples were heated for 30min in a boiling bath at 100^0C.

The sample solutions were left to cool for an hour and 1ml of deionized H_20 was added to each sample. Due to a reaction between acid and water, the solution color changed and heat was produced. A spectrophotometer (PHERAstar plus, BMG LABTECH, Offenburg, Germany) was used to take absorbance values for EW load. Samples were loaded into a clean and flat bottom microplate (Greiner Bio-One, Monroe, NC, USA) containing 96 wells. The optical density was kept at 590nm and the obtained wax values were converted into mg/dm^2 using Beer-Lambert formula proposed by (Ebercon et al., 1977). A standard curve was derived using the sorghum and carnauba wax as both have a positive correlation. Fig. 1 represents the standard curve used for conversion of sorghum wax into mg/dm^2.

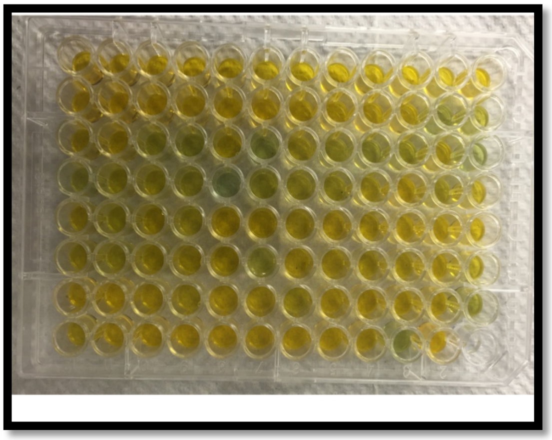

Fig. 3.2. Extracted wax samples in microplate

3.2.6 Statistical analysis

The statistical analysis was performed using JMP Pro 11.2.1 (JMP version 11, SAS Institute Inc., Cary, NC) software. The epiculticular wax values were tested for normality using Shapiro-Wilk test (Ott and Longnecker, 2008). A simple Pearson's correlation was done to check the linear relationship, and the regression procedure was used between leaf epiculticular wax and spectral indices related plant health and growth for low and high EW genotypes. A linear regression analysis was also done for the yield and epiculticular wax relationship.

3.3 Results and discussions

3.3.1 Canopy spectral reflectance using hyperspectral imagery and wax correlation

The results of this study showed a significant difference in the epiculticular wax content among groups of consistently low and high wax lines. The high wax recombinant lines showed a significant increase during the flowering stage and then gradually decreases during grain filling and maturity stages, keeping high wax compared to low wax lines. The comparison between consistently low and high wax lines were significantly different from each other regarding the wax content at different developmental growth stages (fig.3.2). The epiculticular wax and their chemical constituent's varies with the plant and leaf age (Baker and Hunt, 1981). These developmental changes in the leaf epiculticular waxes could be due to variations in their basic chemical constituents including alcohols and alkanes, but these chemical compositions of wax vary greatly from species to species (Wang et al., 2015). The degradation of wax occurs after the flowering stage, where the wax content decreases during the grain filling and maturity stage respectively.

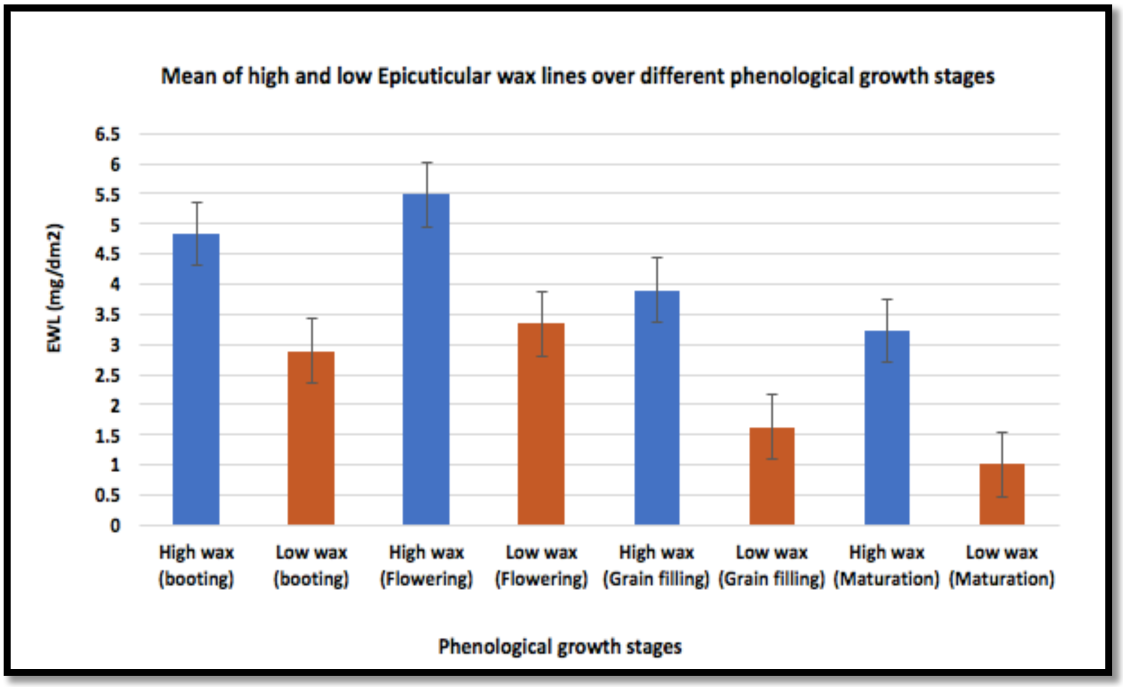

Fig.3.3. The mean of high and low epiculticular wax load lines at booting, flowering, grain filling and maturity stages.

Differences were observed in reflectance pattern between the lines across the photosynthetic active region and the near infrared region. The hyperspectral indices used for estimation of biochemical content of plants were conducted nondestructively. The presence of epiculticular wax affects the spectrum properties of leaf surface, which ultimately affects the performance of the spectral indices to estimate the biochemical content and health of the plant (Lu, 2013).

The lines with consistently more and less wax were measured for their carotenoids content, plant greenness, water content and photochemical reflectance. A marked difference was found in their pattern. The reflectance index tends to decrease in the case of carotenoids pigments, with the age of plants and increase wax content (Fig.3.3). Water band index does not seem to be particularly affected by the presence of epiculticular wax and is considered a more robust estimation of the water content. The plant senescence reflectance index increased with a decrease of epiculticular wax during the vegetative stage of plant development and continued with plant extended maturation compared to high wax genotypes (Fig.3.3 & Fig.3.4).

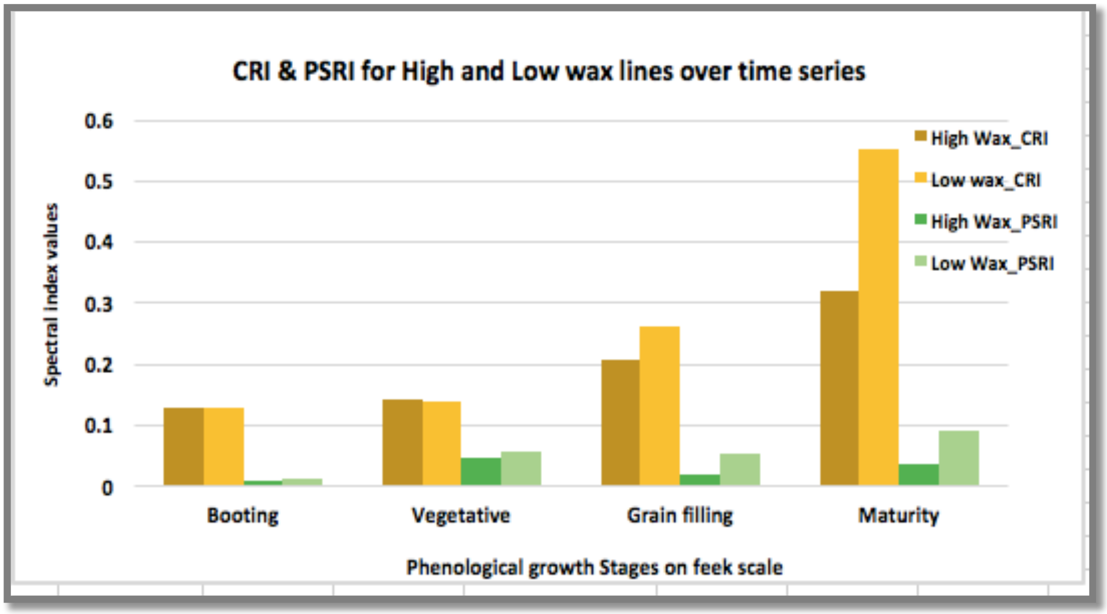

Fig.3.4. CRI & PSRI of consistently high and low wax recombinant lines at different phenological growth stages of plant development.

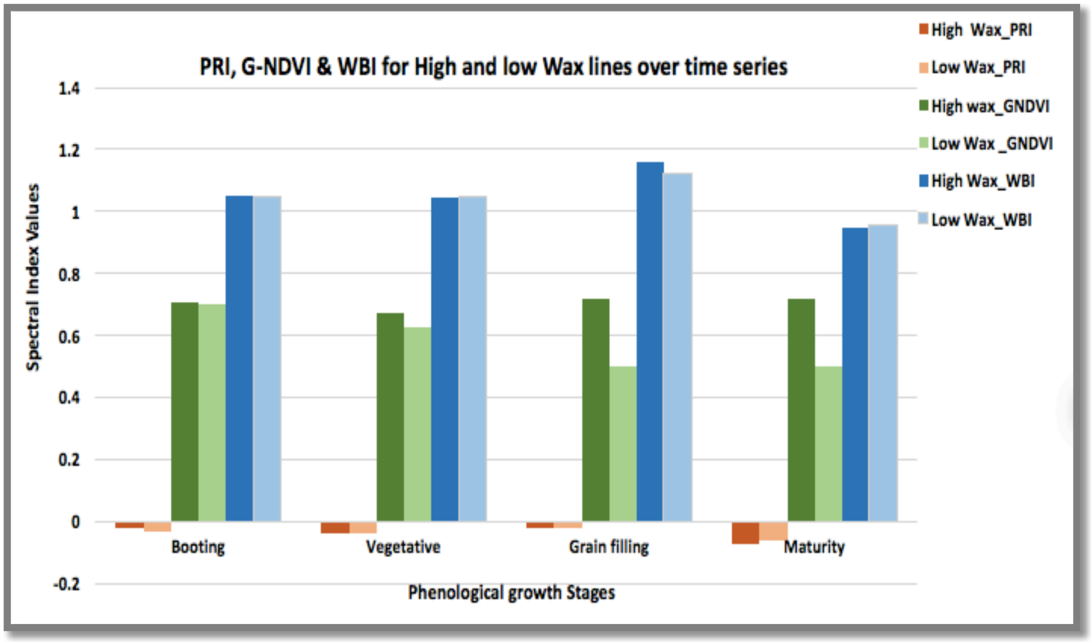

Fig. 3.5. PRI, GNDVI and WBI of consistently high and low recombinant lines at different phenological growth stages of plant development.

The PRI values did not vary significantly among high wax lines, but during the maturation stage it increased as the carotenoid reflectance index decreases. The presence of higher epiculticular wax causes higher reflectance as confirmed by Mondal (Mondal, 2012) indicating their high radiation use efficiency. All spectral indices showed variations between the high wax inbred lines except for the water band index, which did not vary significantly with a correlation value of -0.5, -0.4 at booting and vegetative growth stages, respectively. A correlation of 0.7 was observed during the grain filling stage between high leaf wax and WBI. These values indicated that during the grain filling stage of development the recombinant lines had maintained a good water status in relation to the leaf epiculticular wax and are more active photosynthetically (Table.2). The SI-related vegetation increases with an increase in EW, which indicates the overall plant health and growth for RILs with higher leaf wax content.

Traits	HW_Booting	HW_Flowering	HW_Grain filling	HW_Maturation
CRI	0.76**	0.67*	-0.70*	-0.89**
PRI	0.51	0.62*	-0.67*	-0.74*
PSRI	-0.52^{NS}	0.51^{NS}	-0.52^{NS}	-0.66*
GNDVI	0.73*	-0.57^{NS}	0.62^{*}	0.56^{NS}
WBI	-0.50^{NS}	-0.47^{NS}	0.70*	-0.4^{NS}

Table 2. Pearson’s correlation table of high epiculticular wax lines and plant vegetation indices at booting, flowering, grain filling and maturation stage. *, **, significant at p ≤ 0.01 and 0.05 respectively. NS represents not significant.

Traits	LW_Booting	LW_Flowering	LW_Grain filling	LW_Maturation
CRI	0.45^{NS}	0.55*	0.2^{NS}	0.42*
PRI	0.05^{NS}	-0.17^{NS}	-0.5^{NS}	-0.08^{NS}
PSRI	0.40^{NS}	0.58*	0.61*	0.76*
GNDVI	0.36^{NS}	0.64^{NS}	-0.53^{NS}	0.62^{NS}
WBI	0.36^{NS}	0.15^{NS}	-0.50^{NS}	-0.52^{NS}

Table 3. Pearson's correlation table of low epiculticular wax lines and plant vegetation indices at booting, flowering, grain filling and maturation stage. *, ** significant at $p \leq 0.01$ and 0.05 respectively. NS represents not significant.

The carotenoid reflectance index is sensitive to carotenoids pigments is designed based on measuring stress related pigments present in vegetation. The concentration of carotenoids which is a group of alpha-carotene, beta-carotene and xanthophyll pigments gets increases in weakened plants. This index showed a significant negative correlation with the presence of high epiculticular wax. At the booting stage the correlation of (r=0.869) was significantly higher, which continued to 0.5, -0.7 and -0.9 during vegetative, grain filling and maturity stages, respectively (table.2). The presence of epiculticular wax causes a decrease in the concentration of carotenoids and protecting plants photosynthesis from excessive light energy. These pigments indicate the presence of vegetation stress or the onset senescence before it is noticeable using the unaided eye. The absorption region in the 510 nm is sensitive to total carotenoids concentration relative to chlorophyll content in the 550 nm. The values for green vegetation ranges from 1 to 12 but the number can reach to 15 (Gitelson et al., 2002c).

A stronger correlation was found between low EW lines and PSRI at the extended maturation stage, with a correlation value of 0.62. This index is associated with canopy stress as this index is sensitive to the ratio of bulk carotenoids consisting alpha and beta carotene. The increase in this index is associated with the low wax content which ultimately results in high PSRI values. The common range for this index varies from -1 to 1 with a green vegetation range from -0.1 to 0.2. The photosynthetic light use efficiency is an indicator of any short term changes in the process of photosynthesis and is related to the xanthophyll pigment cycle without any changes in the PAR canopy scale (Filella et al., 1996). The PRI values for high leaf wax RILs were lower compare to the low wax lines, which indicated an increase in their radiation use efficiency. The PRI reflectance measurements are sensitive to carotenoids pigments particularly xanthophyll pigments indicating the carbon dioxide uptake by vegetation with an absorbance range in the 531nm. The common range for PRI varies from -1 to 1.

The amount of water was measured using water band index which depends on many factors including plant architecture, species and the water requirement at different phenological growth stages. An increase in WBI indicates high water content in vegetation. The common range for this index is 0.8 to 1.3. A good indication of WBI is sensitive to absorption in the 970nm compare to 900nm range. The WBI and did not showed any correlation with high waxy lines and it was same for low waxy lines and thought to be more robust estimation of water content.

The electromagnetic spectrum is divided into three main regions: the visible range or photosynthetically active region, the near infra-red region and the mid infrared region. The PAR is associated with the energy production to be utilized in the process of photosynthesis. The incident light on plant leaf surfaces is influenced by the presence of pigments, biochemical structures and water content as they absorb the incident light necessary for photosynthesis. The presence of surface wax greatly enhances the surface reflection (Cameron, 1970). The plant leaf surface provides an important medium for light reflection. The leaf reflectance in the visible range was more affected by the biochemical pigments such as chlorophyll and xanthophyll. The near infrared is mostly associated with the canopy cell structures; however, it has a weak association with water content. Leaf pigments including the carotenoid reflectance index decreases slowly compare to the chlorophyll content and absorbs light strongly in the blue spectral region at 445 nm (Sims and Gamon, 2002), while anthocyanin absorbs in the green region at 550 nm of the electromagnetic spectrum. The absorption increases as the plant gets dehydrated and thus reflectance from plant surface decreases. The presence of the xanthophyll cycle dissipates excess energy and prevents any damage to the photosynthetic apparatus. Any variation present in the pigment concentration provides plant physiological information (Sims and Gamon, 2002).

3.3.2 Sorghum grain yield and wax correlation

All plant surfaces are usually covered with an epiculticular wax layer present in different chemical structures and acting as an important edge between plant aerial organ and the environment (Jenks and Ashworth, 1999). It is reported that leaf waxes are related to grain yield in sorghum (Jordan et al., 1984) and also in other cereals. Proper environmental and agronomic practices help to achieve high grain yield, an important trait in crops. It is a combination of physiological processes that occur during plant developmental growth stages, including the vegetative growth phase, flowering stage, grain filling and maturity stages, and interaction with the environment (Yoshida, 1972). In this study the EW was positively correlated with the final grain yield. The recombinant lines with more wax showed significantly higher yield compare to the lines with low wax. The grain yield varied among high wax and low wax lines with minimum yield of 18.49 and maximum yield of 48.78g. The mean for yield was 29.230 with the standard deviation of 6.64. Regression analysis were carried out between EW and grain yield among high wax line.

A significant correlation was found for high wax lines and yield with an r value of 0.70 at significance level of 0.05 (Fig.3.6), while the r value for low wax lines was 0.62 and showed no significant difference at the significance level of 0.05 (Fig.3.7). These results indicate that the presence of higher EW contribute towards better grain yield in sorghum. A smaller difference was found in grain yield when high waxy lines were compared with the low wax recombinant lines. This indicated the importance of this trait for the selection of a more stable yield, as previous studies showed in wheat (De Vita et al., 2007). Epicuticular wax improved the yield under both water deficit and well-watered conditions (Clarke and Townley-Smith, 1986). Results of this study and previous work strongly suggest that EW would confer a degree of tolerance to sorghum lines and help to maintain yield stability throughout the developmental processes, from booting to the extended maturation stage.

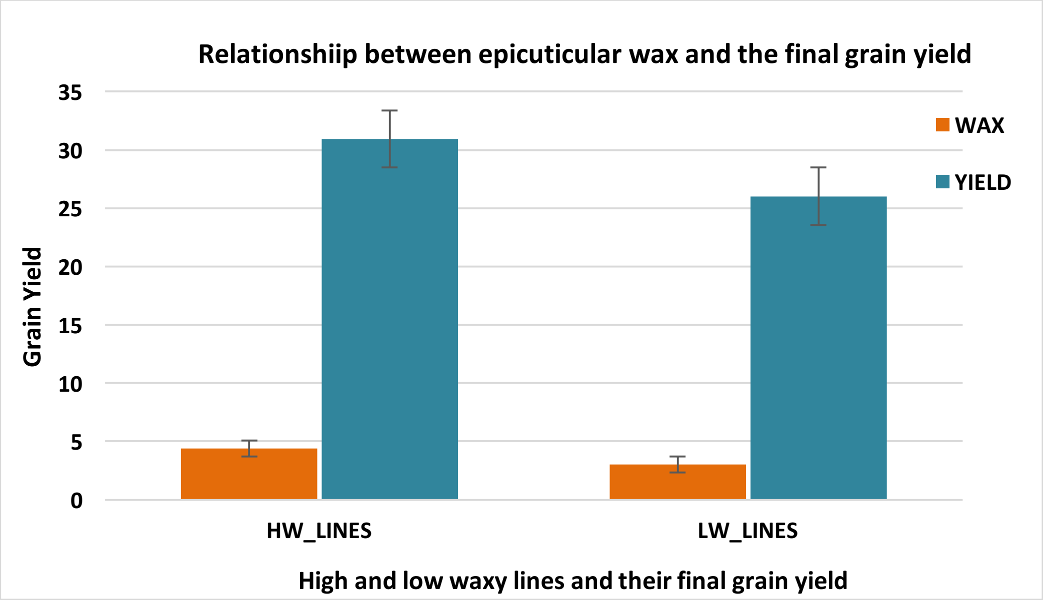

Fig.3.6. The mean high wax lines significantly correlating to the final grain yield compared to the low wax lines.

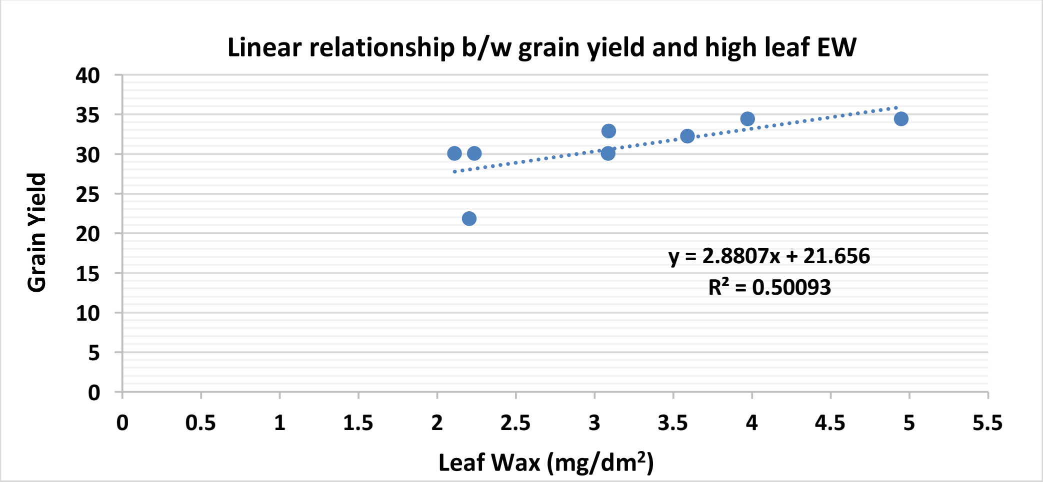

Fig.3.7. A linear relationship between high leaf epiculticular wax and grain yield. A significant correlation was found with an r value of 0.70 at significance level of 0.05.

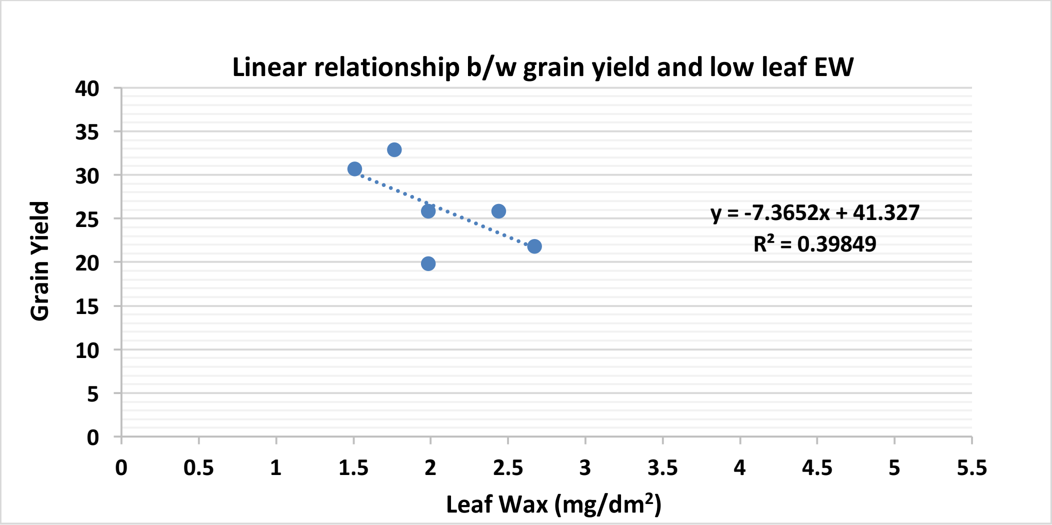

Fig.3.8. A linear relationship between low leaf epiculticular wax and grain yield. A weak correlation was found among low wax lines and yield with a r value of 0.62.

3.4 Conclusions

The presence of epiculticular wax influences the reflectance from leaf surface which depends on presence of leaf pigments including carotenoids, photosynthetic light use efficiency biochemical structures and water content as they absorb the incident light necessary for photosynthesis. The absorption increases as the plant gets dehydrated and thus reflectance from plant surface decreases. Our results suggest that the high wax lines the CRI and PRI values decreases indicating the low concentration of stress related pigments thus improving plant health and extended maturation. The high waxy lines decreases for PSRI and reduces canopy stress at grain filling and maturation growth stages. Our results also relate to the previous studies that a positive correlation between high EW and yield was found. The presence of EW plays important role in preventing yield loses under drought conditions.

CHAPTER IV

SUMMARY

Drought is a major contributing factor to reduced crop productivity and yields. There is an urgent need to adapt drought avoidance mechanisms for the development of resilient crop genotypes. Work has been done to address this issue, but less attention has been given to utilization of physiological attributes to improve crop productivity and water use efficiency. *Sorghum bicolor (L.)* Moench is the world's fifth most important cereal and a source of food for around 750 million people worldwide (Joint, 2011). It can cope with water shortage, yet long-term stress affects sorghum growth and productivity. The presence of epicuticular wax (EW) on the vegetative body of sorghum is one effective mechanism executed for drought resistance. The heavy wax covering is termed "bloom," while bloomless types lack EW layer. The waxy cuticle protects the leaves and reduces evapotranspiration (Ramatoulaye et al., 2016). Our results suggest that that the bloomless has equal potential to accumulate biomass under water stress conditions compared to its bloom counterpart. Water use efficiency of bloom is significantly higher under well-water conditions, where the presence of wax explains the resistance of sorghum to water loss. Under water-stressed conditions, the crops prevent the excessive water loss and the EW increases as described by Jordan, et al. (Jordan et al., 1984; Premachandra et al., 1992). The accumulation of EW under water deficit conditions reduces leaf water potential, which results in increased photosynthetic activity of plants. In this study, high resolution hyperspectral vegetation indices were used to monitor the influence of EW on plant water uses biochemical content and health. The presence of epiculticular wax influenced the reflectance from leaf surface which depends on presence of leaf pigments including carotenoids, photosynthetic light use efficiency biochemical structures and water content as they absorb the incident light necessary for photosynthesis. Our results also suggest that the high wax lines the CRI and PRI values decreases indicating the low concentration of stress related pigments thus improving plant health and extended maturation. The high waxy lines decreases for PSRI and reduces canopy stress at grain filling and maturation growth stages. Our results also relate to the previous studies that a positive correlation between high EW and yield was found. The presence of EW plays important role in preventing yield loses under drought conditions.

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