MOLECULAR MAPPING OF TRAITS IN COWPEA

A Dissertation

by

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ABSTRACT

Cowpea (*Vigna unguiculata* L. Walp) is a leguminous crop that many around the world rely on to meet their basic nutritional needs through the consumption of the protein and fiber rich grain and vegetative matter. Water and soil fertility stress affect this crop like many other crops; by reducing biomass and grain yield. Genotypes of cowpea have been identified that possess genes that confer tolerance to drought and resistance to interveinal chlorosis (IC) due to micronutrient deficiency. In order to map the quantitative trait loci (QTL) associated with these traits, a recombinant inbred line population (RIL) was created from the parental genotypes 'IT98K-476-8' and 'Golden Eye Cream' (GEC). This population was phenotyped for vegetative and yield traits and leaf chlorosis. The vegetative and yield data was used to calculate a drought susceptibility index (DSI) that was used in QTL mapping. The population was genotyped by restriction site-associated DNA sequencing (RAD-seq), and a linkage map spanning 1,084 cM was constructed from 4,154 single nucleotide polymorphisms (SNPs). Mapping for additive QTL identified 78 QTL that met a LOD threshold of three and 13 QTL that met the permuted LOD threshold for various traits and one DSI. Epistatic mapping resulted in 95 QTL involved in digenic interactions. From correlative statistics and co-localizations of QTL, it can be concluded that many of the traits mapped here are regulated by the same genes and that there are pleiotropic tradeoffs between some traits.

DEDICATION

To my family, who passed the genes for love of plants onto me.

ACKNOWLEDGEMENTS

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1. INTRODUCTION

All chapters in this dissertation utilize, with permission, unpublished raw data collected by Brijesh Angira.

There are many factors that adversely affect crop production. Among these factors are abiotic and biotic stresses. Abiotic stresses include temperature, soil fertility, and water stress. The biotic stresses include insects, fungal, viral and weed pests. Of the abiotic stresses, drought stress or the lack of adequate usable soil moisture, is the most limiting factor to crop yields worldwide, and the expanse of drought affected crop land is predicted to widen with global climate change (Rosenzweig et al. 2001).

Twenty percent of the world, on average, is in a state of drought and this percentage is rising. Thirty-five percent of the Earth is projected to experience drought by the year 2020. Drought stress reduces plant biomass, seed set (Herrero and Johnson 1981) and seed weight (Desclaux et al. 2000) which all ultimately result in lower yields (Jaleel et al. 2009). Water stressed plants are frequently the target of insects (Mattson and Haack 1987) and pathogens (Desprez-Loustau et al. 2006) and therefore plants often suffer from multiple types of stress at once, which compounds the effects on yield. Reduced yields result in less return for the farmer and increased food prices for the consumer (Bar-Yam et al. 2015). Increased prices for the consumer mean less consumption. This decreased consumption can lead to hunger and malnutrition (Wu et al. 2014).

Soil fertility stress is another stress that plants and farmers often face. Fertility stress can come in the form of deficient supplies of nutrients, overabundance of elements, or factors that cause the availability of elements to be over available to the point of toxicity, or soil bound and unavailable. Soil pH is one of these factors. Alkaline or calcareous soils make up 30% of arable lands (Hell and Stephan 2003). Soils with a high pH do not allow for solubility and uptake of many micronutrients including copper (Cu), iron (Fe), and zinc (Zn) (Kumar et al. 2016). Acid soils, or soils with a low pH, can free up many of these elements which are soil bound at a high pH, but also bind some essential minerals such as calcium (Ca), phosphorus (P) and manganese (Mn). Aluminum (Al), which is phytotoxic, becomes available for uptake

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at low soil pHs (Fan et al. 2015). All of these nutrient deficiencies and toxicities can ultimately lead to diminished yields.

When dealing with drought stressed environments and soil fertility issues, there are some options available to alleviate the issue. The options often involve changing the environment or changing the crop that is farmed. One way to alleviate plant stress on a drought stricken field, is to add water via irrigation. Alleviation of stress caused by acidic soil can be made possible through the incorporation of agricultural lime which can be applied to raise the pH to an optimal level (Huggins et al. 2015). Elemental sulfur (Su) can be applied to an alkaline soil to lower the pH (Gupta and Abrol 1990). Many times changing the environment is not a feasible option though. Many farmers in arid lands do not have access to irrigation water, or this option is too expensive to justify. Sulfur, and its incorporation, also incurs a cost the farmer.

An alternative option to managing soils and soil water status, is to change the crops being grown. There are many crop options available that are suited to semi-arid environments and therefore will thrive in areas that receive less precipitation (Hall et al. 1979). There are also crops that are suited to more alkaline or acidic soils (Cakmak et al. 1994) (Clemens 2001).

Drought tolerance is most simply defined as the ability to maintain yield under water stress (Clarke et al. 1992). There are crops that are more able to maintain yield under water stress than others. Some known drought tolerant crops are sorghum (Paterson et al. 2009), amaranth (*Amaranthus* spp.) (Liu and Stützel 2004) (Johnson and Henderson 2002) and cowpea (*Vigna uguiculata* L. Walp) (Hall et al. 1979), to name a few. Within a crop species, genotypes exist that are more tolerant than others. Once these genotypes are identified, they can be crossed with other genotypes to ultimately create a line or hybrid that contains all of the desired agronomic traits plus the drought tolerance.

Cowpea is a crop in which extensive work has been done in identifying genotypes that are more drought tolerant (Singh and Matsui 2002). Cowpea, also known as black eyed pea, purple hull pea or crowder pea, is an annual crop that has an important role in cropping systems in Africa. Due to its ability to fix nitrogen, shade tolerance, and the short maturity time, it can be used in rotation or intercropped with other crops (Horst and Härdter 1994) (SARE 2012a) (Singh 2014).

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The grain of the cowpea can be eaten fresh or stored and dried for later use. The vegetative matter of the plant can also be consumed. Both the grain and vegetative matter of the cowpea plant are important in the diet of many Africans, especially those that are vegetarians. The grain is nutrient dense, containing approximately 25% protein and 4% fat. The grain also boasts high concentrations of sodium (Na), potassium (K) and P compared to other legumes (Iqbal et al. 2006). Like most legumes, the cowpea grain also contains dietary fiber (Messina 1999). In developing countries, legumes, on average, contribute 7.5%, of the protein to the human diet, but in the poorest countries they contribute up to 10%, compared to 2.5% in developed countries (Akibode and Maredia 2011). Cowpea leaves, which are also consumed, contain approximately 34% protein, and are a good supply minerals such as K, P and Ca and phytochemicals such as carotenoids (Imungi and Potter 1983) which have been shown to play important roles in human health (Rao and Rao 2007).

Cowpea is grown in areas around the world, but most of the production occurs in the continent that it is native to, Africa. In 2000, Africa accounted for 68% of the world's production, with Brazil in second at 17%. The United States accounted for 2% of the production. In Africa, Nigeria and Niger accounted for the majority of the production, with Tanzania and the Democratic Republic of the Congo also producing notable amounts. From 2010 to 2014, Nigeria produced on average approximately 3.39 million tons of cowpea, while Niger produced approximately 1.6. The production of cowpea around the world has been rising, from 2.75 to 5.6 million tons from 2008 to 2014 (FAO 2015). Among legumes, cowpea accounts for 18% of the area in production, which is less than chickpea (*Cicer arietinum*) at 18% and dry bean at 46% (Akibode and Maredia 2011).

Because cowpea is native to the semi-arid climates of Africa, it has evolved mechanisms that allow it to be tolerant to drought. Mechanisms of drought tolerance can be grouped in three categories; drought escape, tolerance, sometimes termed resistance, and avoidance (Mitra 2001; Yue et al. 2006). It is of note that mechanisms of tolerance are not mutually exclusive and plants can use multiple mechanisms to ensure survival during drought (Agbicodo et al. 2009). Plants with escape mechanisms are able to complete their life-cycle fast enough to avoid the damaging effects of drought. In cowpea, early maturing genotypes that can reach maturity in under 65 days can be used to fill this niche (Hall 2004). Tolerance is

characterized as the ability of a plant to function with low tissue water potential. Osmotic adjustment through solute accumulation is one method a plant uses to tolerate drought status. An alternative tolerance type mechanism of cowpea is senescence and abscission of leaves during drought so that tissue water in the stem is maintained and the plant can continue with its lifecycle once water has been restored (Gwathmey and Hall 1992). Avoidance mechanisms allow the plant to maintain more optimal tissue water status during times of low soil water moisture and can also be thought of a desiccation avoidance. Root systems that penetrate deeper into the soil profile and have ideal architecture are thought to aid in drought avoidance (Mitra 2001) (Matsui and Singh 2003). Mechanisms of avoidance in cowpea also include increased water use efficiency (WUE), reduced stomatal conductance, reduced leaf area (Anyia and Herzog 2004) and altered leaf orientation or paraheliotropism (Schakel and Hall 1979).

Two tolerance types have been further described in cowpea; Type 1 and Type 2 tolerance (Mai-Kodomi et al. 1999). In Type 1 tolerance, the unifoliates, which are the lowest set of leaves, will desiccate around the same time point as the trifoliates. Unifoliates desiccate earlier than trifoliates in Type 2 tolerance, and it is believed that this mechanism allows for a reduced sink at the lower region of the plant so that it can partition resources to growth to the terminal end. Type 1 and Type 2 genotypes have shown no difference in overall plant water status though (Verbree et al. 2014) and unifoliate wilting has been shown to not correlate with other drought tolerance traits (Muchero et al. 2008).

Drought tolerance studies are often conducted in the field to look at the effect of drought on yield. Greenhouse experiments that evaluate the plants at a vegetative stage have also been conducted. The advantage of greenhouse studies that only evaluate plants at the vegetative stages, is that less space is needed and therefore larger numbers of genotypes can be screened. When screening seedlings, time to maturity and photoperiodism is not a factor that can confound results because yield is not obtained, assuming that time to maturity or photoperiodism does not affect growth at juvenile stages. Uniform soil water status is able to be maintained throughout all entries when genotypes are screened for drought tolerance using the box method proposed by Singh (1999).

Many genetic studies have been performed in order to better understand the underlying mechanisms of drought tolerance in cowpea. Of the molecular mechanisms, enzymes involved in abscisic

acid biosynthesis (Iuchi et al. 2000) and upregulation of ascorbate peroxidase (D'Arcy-Lameta et al. 2006), and glutathione reductase (Contour-Ansel et al. 2006) have been identified. The gene action of leaf and stem desiccation has also been studied (Mai-Kodomi et al. 1999) (Verbree et al. 2015).

An approach that needs no prior knowledge of the genes underlying the mechanisms of tolerance is quantitative trait locus (QTL) mapping. QTL mapping is a strategy that incorporates phenotypic data with genotypic data in order to identify regions of the genome associated with the trait of interest. The incorporation of data is done through advanced statistical software and when the input is of good quality, will result in a map that has the trait of interest localized to a region or regions of the genome. Once quantitative trait loci are mapped, the region associated with QTL can be cloned for further analysis. Markers for marker assisted selection (MAS) can also be identified (Collard et al. 2005).

Mapping studies have been carried out in order to identify QTL associated with drought tolerance in cowpea. Muchero at al., identified QTL associated with grain yield (Muchero et al. 2013), drought induced senescence, and maturity (Muchero et al. 2009b). From these studies it is evident that many of the genes that confer drought tolerance co-localize.

Less work has been done identifying cowpea genotypes that are tolerant to alkaline soils even though it is known to be sensitive. Cowpea often becomes yellowed when grown in soils with a pH over 7.5 and this yellowing is termed interveinal chlorosis (IC). Interveinal chlorosis is due to the inability to incorporate micronutrients that are necessary for chlorophyll formation and other metabolic processes (Brown 1956) and therefore, in cowpea, high pH tolerance is defined as the ability to maintain yields at a pH of 7.5 (Goenaga et al. 2010).

Soybean (*Glycine max*) is a related legume of economic importance that also is adversely affected by alkaline soils, yet genotypes have been identified that are more tolerant to alkaline soils (Fehr 1982). Genetic studies on soybean have had conflicting conclusions, with some concluding that resistance to chlorosis is a single gene trait (Weiss 1943) and others finding that it is a multi-gene trait (Lin et al. 1997). Since iron is often the most limiting nutrient in alkaline soils, many studies focus on the genetics of acquisition and transport of this metal when considering tolerance to alkaline soils. Considering that there are many steps in iron transport, from solubilizing Fe^{3+} in the root zone to reducing it to the Fe^{2+} form,

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then transport by iron transporters, one could conclude that it is a multigene trait. The plant may also enable mechanisms such as root hair formation and phloem citrate concentrations to better access and transport the iron, which would further the evidence of it being a multi-gene trait (Hell and Stephan 2003). The case for major and minor genes can be made for genetic regulation of plant iron status. The genes in *Arabidopsis thaliana,* IRT1 and IRT2, have been identified as iron transporters, with knockout mutants of IRT1 being lethal to the plant, thus its designation as a major gene. IRT2 has also been proven to be important, though not as detrimental to plant survival when knocked out. Homologs of these genes have been discovered in other species such as pea (*Pisum sativum*) and tomato (*Solanum lycopersicon*).

It is not enough to just know about the function or inheritance of genes. With the world's population projected to reach 9 billion by 2050, breeders need to utilize these stress tolerance genes in their breeding programs in order to create genotypes that can help fill the gap between food supply and demand on the same amount of farm land (Godfray et al. 2010). Conventional breeding for drought tolerance traits can be challenging due to their quantitative nature (Agbicodo et al. 2009).

Marker assisted selection (MAS) has been proven to increase the efficiency of breeding for many traits (Xu and Crouch 2008) including drought tolerance (Schneider et al. 1997). In MAS, genetic markers linked to the gene of interest or quantitative trait loci must first be discovered. To do this the QTL must first be mapped to the genome. The genetic markers linked to the trait can then be used as a selection tool. Genotypes that possess these markers can then be selected. These genotypes can then be used in research with the goal of determining the effect of the gene on the plant's phenotype, or the genotype can be used in future breeding efforts.

MAS can also be used to identify genotypes that contain tolerance genes so that breeders can utilize them faster and thus help to feed the world's growing population. Due to the synteny of cowpea with crops such as soybean and alfalfa (*Medicago trunculata*) (Muchero et al. 2009a) the markers developed for cowpea can be helpful in breeding other crops of agronomic importance.

The long term goal of this project is to develop the tools needed for breeding genotypes of cowpea that exhibit superior drought and interveinal chlorosis resistance along with traits of other grower and consumer importance. *The primary objective* of this dissertation is to identify some genetic loci that underlay

drought tolerance and IC resistance. The *central hypothesis* of this objective is that tolerance mechanisms play an important role in determining the yield of the crop under stress and these mechanisms are under genetic regulation and are heritable and transferrable to other genotypes. I further hypothesize that when tolerance genes are optimized, higher yields will be achieved.

In this dissertation, I have tested my hypothesis using the following specific objectives:

Objective 1) Identify traits associated with drought tolerance/susceptibility and interveinal chlorosis resistance in a recombinant inbred line (RIL) population of cowpea.

Objective 2) Correlate the traits associated with drought tolerance and interveinal chlorosis resistance to loci on the cowpea genome. Determine the effect of the quantitative trait loci on the phenotype.

2. MAPPING QTL ASSOCIATED WITH DROUGHT SUSCEPTIBILITY IN COWPEA

2.1 Introduction

Twenty percent of the world, on average, is in a state of drought at any given point in time and this percentage is rising. By the year 2020, 35% of the Earth is projected to be experiencing a drought period. Drought stress reduces plant biomass, seed set (Herrero and Johnson 1981) and seed weight (Desclaux et al. 2000) which all ultimately result in lower yield (Jaleel et al. 2009). Water stressed plants are frequently the target of insects (Mattson and Haack 1987) and pathogens (Desprez-Loustau et al. 2006) and therefore plants often suffer from multiple types of stress at once, which compounds the effects on yield.

Drought tolerance is most simply defined as the ability to maintain yield under water stress (Clarke et al. 1992). There are crops that are better able to maintain yield under drought stress more than others, and cowpea (*Vigna uguiculata* L. Walp) is one of these crops (Hall et al. 1979). Cowpea, also known as black eyed pea, purple hull pea or crowder pea is an annual crop that has an important role in cropping systems in Africa. Due to its ability to fix nitrogen, shade tolerance, and the short maturity time, it is often used in rotation or intercropped with other crops (Horst and Härdter 1994) (SARE 2012a) (Singh 2014). The grain of the cowpea can be eaten fresh or stored and dried for later use. The vegetative matter of the plant can also be consumed. Both the grain and vegetative matter of the cowpea plant are important in the diet of many Africans, especially those that are vegetarians, and because this crop is native to the arid and semi-arid areas of Africa, many genotypes have evolved genes that confer drought tolerance. Because cowpea can survive and yield in environments that other crops cannot, extensive work has been done in identifying genotypes that confer a superior drought tolerance (Singh and Matsui 2002) (Verbree et al. 2015).

The population of the world is projected to reach 9 billion by 2050. In order to meet the nutritional demands of the population, growers must be able to produce more on the same amount of land

(Godfray et al. 2010) under more stressful conditions. The first step in making this possible is by breeding genotypes that are higher yielding under stress. Marker assisted selection (MAS) is tool known to increase the speed and accuracy of breeding so that these genotypes can be created more efficiently and effectively.

MAS has been proven to increase the efficiency of breeding for many traits (Xu and Crouch 2008) including drought tolerance (Schneider et al. 1997). In MAS, genetic markers linked to the gene of interest or quantitative trait locus must first be discovered. To do this, the QTL must first be mapped to the genome. The genetic markers linked to the trait can then be used as a selection tool. Genotypes that possess the linked markers can then be selected or deselected. Chosen genotypes can then be used in research with the goal of determining the role of the gene in the plant's phenotype, while the genotype can be used in future breeding efforts.

Mapping studies have been undertaken that have identified QTL associated with drought tolerance at the seedling stages up to maturity (Muchero et al. 2009b; Muchero et al. 2010). These studies have only looked at additive genes though, while the possible epistatic interactions that can account for a large percent of the variance in a trait, have yet to be studied. Knowledge of these epistatic interactions would be useful in understanding the complex mechanisms of drought tolerance.

The goal of this study was to map the additive and epistatic QTL associated with various traits including yield traits in a RIL population derived from the cross of two cowpea genotypes that differ in their mechanisms of drought tolerance. The knowledge gained and markers identified in this study may aid in future efforts in cowpea, and due to the synteny of cowpea with crops such as soybean and alfalfa (Muchero et al. 2009a).

2.2 Material and Methods

  2.2.1 Population

Two genotypes of cowpea, 'IT98K-476-8,' an International Institute of Tropical Agriculture (IITA) breeding line and, 'Golden Eye Cream,' (GEC), a line released by the Texas Agricultural Experiment Station (Miller and Scheuring 2006), were identified as having differential tolerance to drought. GEC exhibits an escape mechanism, which is exhibited by achieving its total vegetative growth

and grain yield before water stress becomes a limiting factor. IT98K-476-8 exhibits a drought avoidance strategy by slowing above ground vegetative growth and delaying pod set until the water stress in relieved. These two lines were crossed to produce the F_1. The F_1 was then advanced by single seed descent to the F_8 generation. This F_8 population contained 184 recombinant inbred lines (RILs) which were segregating for traits including plant habit, height and biomass, stem, flower and seed pigmentation, flowering date, seed texture and shape, determinacy and grain yield traits.

2.2.2 Phenotyping

The 184 lines of the RIL population and the parental lines IT98K-476-8 and GEC were planted in College Station, TX (CS) in 2014 and in Corpus Christi, TX (CC) and Weslaco, TX (W) in 2015 between the months of June and July. The lines were randomized and planted in single row, three meter plots with one meter alleys. The field was divided into four reps, with the two control (C) reps receiving supplemental irrigation and the remaining two drought treatment (D) reps not receiving irrigation after stand establishment. The plants in Corpus Christi and Weslaco were given a foliar application of Krystal Clear® Crop Mix to alleviate micronutrient deficiencies. Plots were phenotyped in the field for height (HT) by measuring the length of the main stem from the ground to the shoot tip with a meter stick and for biomass (BM), or the amount of vegetation the plant amassed, by visual scoring from one to ten with one having the least amount and 10 having the most. Number of days from planting to flowering (DTF) was also recorded. Pods were harvested separately from three plants per plot 90 days after planting. Pods from CC and W were weighed to determine the total weight of the pods per plant (TPW). Pods from all locations were counted to determine the number of pods per plant (PPP). The pods were then threshed to obtain the grain, which was then weighed to determine the total seed weight (TSW) per plant. One hundred normal random seeds were weighed to obtain the one hundred seed weight (HSW). The TPW was divided by PPP to obtain the average weight per pod (WTPP). The weather data was accessed from The Crop Weather Program (Texas &M AgriLife Research 2016) and U.S. Climate Data (Your Weather Service 2016) websites. The RIL population was also planted in the greenhouse under 14 hours of light for a separate evaluation of DTF. The plants were planted in one gallon plastic pots, with the two control

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replications receiving enough water to replenish demand and the two drought treated replications receiving exactly half that amount.

By using an equation by Fischer and Maurer (Fischer and Maurer 1978) the drought susceptibility index (DSI) was calculated for each line in the RIL population for each yield trait (Table 1). The equation $DSI= (1-Y_D/Y)(1-X_D/X)$ accounts for genotypic variability in yield potential (Y) and for varying intensities of water stress (WS) in different environments by including the second term $(1-X_D/X)$. This index has also been used in QTL mapping for heat and drought stress in other crops (Du et al. 2009) (Mason et al. 2010).

Table 1 Abbreviation, method of measurement and location of each trait phenotyped

Trait	Abbreviation	Measurement	Location
Days to flowering (days)	DTF	Number of days from planting to first bloom	CS, CC, GH, W
Height (cm)	HT	Length from ground to tip of main stem	CS, CC, W
Biomass	BM	Visually scored as a plot average from 1 to 10	CS, CC, W
Total seed weight (g)	TSW	Weight of seed from a single plant	CC, W
Pods per plant (count)	PPP	The number of pods on a single plant	CS, CC, W
Total pod weight (g)	TPW	The weight of all pods on a single plant	CC, W
Weight per pod (g)	WTPP	Total pod weight divided by pods per plant	CC, W
One hundred seed weight (g)	HSW	Weight of one hundred random seeds	CC, CS, W
Drought susceptibility index	DSI	Calculated for each phenotyped trait	

2.2.3 Genotyping

Five to ten grams of fresh cowpea cotyledon tissue was collected from each RIL and ground with liquid nitrogen. The nucleic DNA was then isolated via a modified Doyle and Doyle method. The normalized DNA was then digested with the enzymes BamHI and MluCI. The DNA was purified to eliminate the BamHI enzyme and then Illumina adaptors ligated, then once more purified. The samples were then multiplexed and then amplified. The PCR protocol is as follows: 98°C for 30 seconds (s), then 15 cycles of 98°C for 10 s, 65°C for 30 s and 72°C for 30 s, followed by 72°C for 5 minutes. The PCR reaction was then purified. The RILS were sequenced via restriction associated DNA sequencing (RAD-

seq) by BGI Americas in Cambridge MA on an Illumina Hi-Seq 2500. The analysis of sequences identified single nucleotide polymorphisms (SNPs) among the RILS and the reduced sequencing allowed for the construction of a linkage map.

2.2.4 Molecular mapping

The linkage map was created using QTL IciMapping v4.0 (Meng et al. 2015). The Kosambi function was used to convert recombination frequency to mapping distance. The markers were first binned to identify redundant markers which were correlated in the RIL population. The bins were then ordered to create the linkage map and rippled to further define the order of the map.

QTL were mapped by using the QTL by environment interactions for multi-environment trials (MET) program in QTL IciMapping v4.1. The function ICIM-ADD was used to identify single effect QTL and ICIM-EPI was used to locate QTL involved in epistatic or digenic interactions. The mean of the data from each plot for each replication in the control replications of the field in each location were used to map all of the traits. The DSI value for each RIL at every location was used in mapping the drought tolerance genes. For each trait and DSI, all locations were mapped together, and a 1,000 permutation test with P=0.05 was used to calculate the logarithm of odds (LOD) threshold. The PIN value for main effect mapping was set to 0.001 and the p value for entering a variable for the epistatic mapping was set to 0.0001.

2.2.5 Statistical analysis

ANOVA of the parental lines and RIL population was done using PROC GLM in SAS v9.4, (SAS Institute., Cary, NC, USA). Variance components and broad sense heritability (H^2) estimates were calculated for both control and drought stress treatments on an entry mean basis using AOV in IciMapping v4.1 which utilizes the formula: $H^2=\sigma^2g/\sigma^2g + \sigma^2ge/e + \sigma^2error/re$. In this formula, the term σ^2g represents the genotypic variance, σ^2ge represents the genotype by environmental variance, σ^2error represents the error variance, e was the number of environments and r was the number of reps per environment. Pearson's correlations were carried out on all yield traits and DSIs for all traits using PROC CORR in SAS v9.4. LSmeans contrasts were performed for QTL with large additive effects using JMP Pro 12.0.1 (SAS Institute., Cary, NC, USA).

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2.3 Results

  2.3.1 Correlations and heritability

          Due to the significant interaction of location on many of the traits among the RIL population

Appendix A), the three locations were analyzed separately for all traits. IT98K-476-8 and GEC did not see

a significant change in DTF among locations except for CS, where the difference was highly significant

for both lines, with IT98K-476-8 and GEC flowering, on average 3.75 and 3.68 days earlier, respectively

(Table 2). The RIL population flowered under drought on average approximately four days later than

control d plants. Plant height had a highly significant reduction under drought for both parents and the RIL

population in all locations, with the exception of a non-significant reduction for GEC in Weslaco and a

significant reduction for IT98K-476-8 in the same location. There was also a highly significant reduction

($P< .01$) for biomass in all locations for both parental lines and RILs with the exception of GEC in College

Station, where there was no significant reduction and in Weslaco, where the reduction was only significant

at the $P=0.05$ level. Total seed weight was decreased under drought in Corpus Christi for both parental

lines and RILs. The RILs were significantly different from each other at the $P=0.01$ level in all locations

for pods per plant, where the average of the RIL population in Weslaco was almost double the amount of

PPP under drought. IT98K-476-8 also had a higher average of pods per plant in CC. The other

environments saw a reduced number of pods set in the drought treatments. Total pod weight was reduced

under drought by almost half for IT98K-476-8 in CC, and this reduction was highly significant while there

was not a significant reduction in total pod weight for GEC in CC. There was an increase in TPW for

IT98K-476-8 in W, though not significant, while GEC had a significant reduction in TPW. The RILS in

both locations had highly significant reductions in TPW under drought. Weight per pod was non-

significant for all locations and parents with the exception of GEC in CC, where WTPP was increased

approximately two fold under drought. The RIL population saw a highly significant reduction in WTPP at

both locations. IT98K-476-8 had a highly significant increase in one-hundred seed weight in CC and W

under drought, but there was no significant effect of the drought treatment in CS. The opposite was seen

for this trait among GEC plants, where there was no affect in CC or W, but there was a significant

decrease in CS. The one-hundred seed weight of all RILs significantly decreased in all locations.
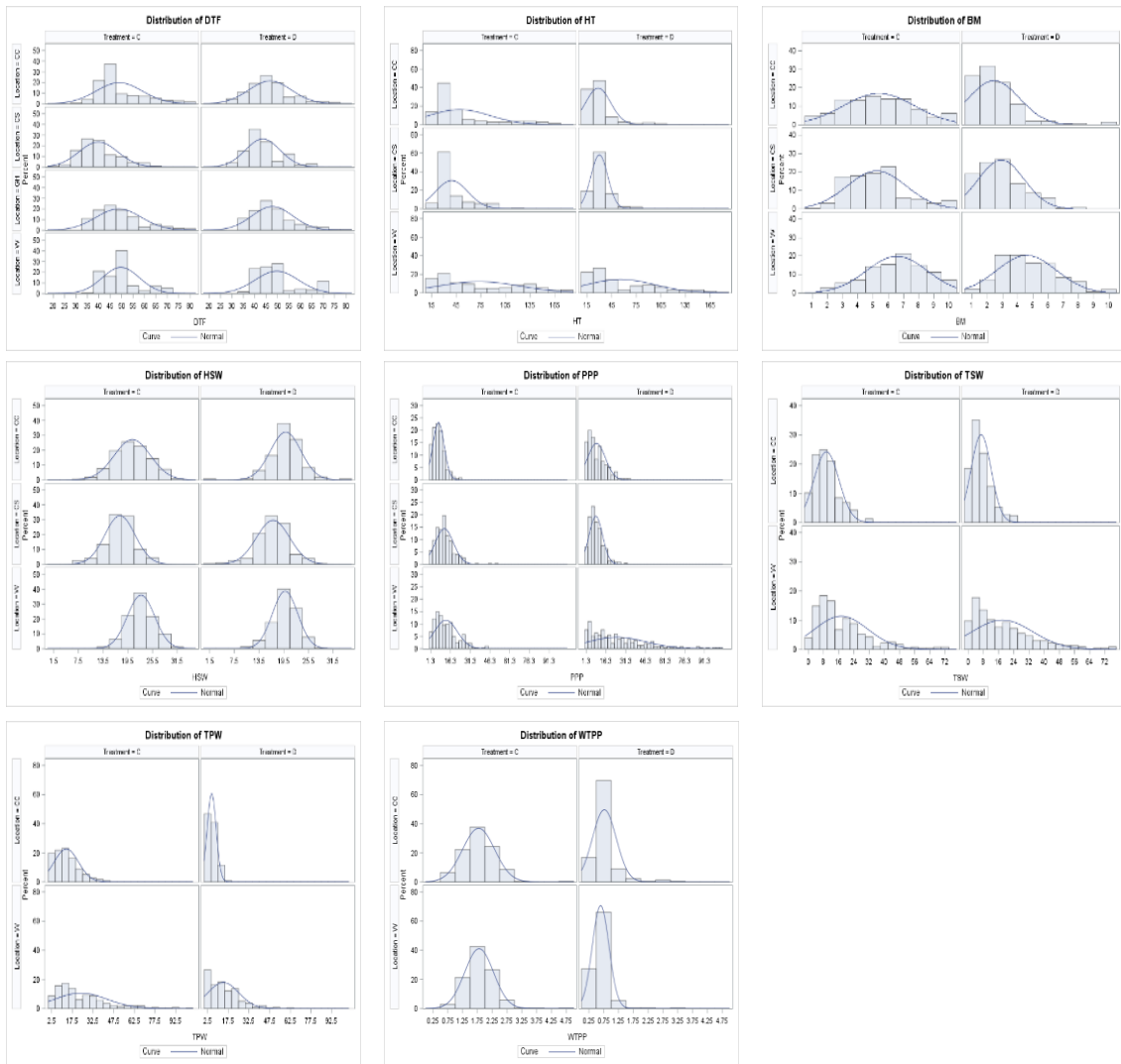
Fig. 1 Histograms of the distributions of all traits measured

Table 2 Means and standard errors for the parental genotypes, means and standard errors for the RIL population, WS value and average DSIs for the RIL population

Trait	Location	476-8 Control	476-8 Drought	GEC Control	GEC Drought	Recombinant Inbred Lines Control	Recombinant Inbred Lines Drought	WS	AVG DSI
Days to flowering (days)	CC	51.38 ± 0.96	54.81 ± 1.90ns	39.75 ± 1.1	39.13 ± 2.25ns	49.13 ± 10.11	46.71 ± 9.32ns		
	CS	46.94 ± 0.95	43.19 ± 0.75**	39.12 ± 0.98	35.44 ± 0.99**	39.78 ± 8.49	43.75 ± 7.59**		
	GH	- ± -	- ± -	- ± -	- ± -	48.71 ± 9.95	47.74 ± 8.94ns		
	W	49.44 ± 0.32	50.31 ± 1.72ns	41.75 ± 0.27	42.94 ± 0.84ns	49.65 ± 8.12	49.49 ± 9.49ns		
Height (cm)	CC	54.21 ± 4.85	29.41 ± 1.45**	24.56 ± 1.29	20.72 ± 1.28*	48.85 ± 36.90	28.49 ± 15.19**	0.70	0.92
	CS	36.02 ± 0.98	30.00 ± 0.94**	30.20 ± 1.18	24.61 ± 0.53**	39.40 ± 19.71	29.93 ± 10.29**	0.34	0.22
	W	175.38 ± 15.2	129.40 ± 13.32*	29.77 ± 2.22	26.50 ± 1.91ns	72.90 ± 49.66	55.92 ± 41.46**	0.17	-0.06
Biomass rating (score 1-10)	CC	7.00 ± 0.22	5.00 ± 0.39**	3.19 ± 0.21	1.94 ± 0.25**	5.38 ± 2.35	2.40 ± 1.67**	0.55	0.78
	CS	5.13 ± 0.59	3.25 ± 0.36**	4.07 ± 1.86	3.07 ± 1.9ns	5.23 ± 1.94	2.91 ± 1.51**	0.45	0.16
	W	7.56 ± 0.18	6.81 ± 0.19**	6.44 ± 0.16	5.88 ± 0.15*	6.56 ± 2.00	4.57 ± 1.96**	0.30	0.74
Total seed weight (g)	CC	8.32 ± 0.68	4.80 ± 0.69**	8.49 ± 1.28	7.30 ± 1.18ns	9.53 ± 6.59	6.78 ± 5.31**	0.29	0.01
	W	10.04 ± 1.58	11.39 ± 2.04ns	22.00 ± 1.39	17.77 ± 1.80ns	17.30 ± 14.05	17.60 ± 15.91ns	-0.11	0.16
Pods per plant (count)	CC	7.67 ± 1.14	4.12 ± 0.63*	13.77 ± 0.89	5.48 ± 0.63**	7.03 ± 4.31	8.90 ± 6.70**	0.22	-0.09
	CS	11.37 ± 2.24	9.52 ± 1.72*	10.59 ± 1.65	6.54 ± 0.87**	11.47 ± 6.95	8.41 ± 5.15**	-0.38	0.02
	W	7.67 ± 1.14	9.58 ± 1.53ns	13.77 ± 0.89	11.99 ± 1.25ns	12.62 ± 8.68	24.62 ± 21.57**	-1.05	2.83
Total pod weight (g)	CC	12.57 ± 0.98	6.54 ± 1.02**	10.34 ± 1.49	8.57 ± 1.33ns	13.21 ± 8.80	5.54 ± 3.29**	0.58	0.21
	W	14.99 ± 2.35	17.86 ± 3.10ns	26.97 ± 1.69	21.42 ± 2.10*	23.73 ± 18.80	13.98 ± 11.15**	0.39	-0.07
Weight per pod (g)	CC	2.07 ± 0.23	1.56 ± 0.07ns	0.70 ± 0.06	1.45 ± 0.09**	1.79 ± 0.54	0.77 ± 0.40**	0.57	0.30
	W	1.91 ± 0.05	1.79 ± 0.06ns	1.96 ± 0.02	1.86 ± 0.08ns	1.81 ± 0.49	0.65 ± 0.28**	0.64	0.40
One hundred seed weight (g)	CC	16.33 ± 0.26	19.95 ± 0.49**	23.99 ± 0.23	22.72 ± 0.71ns	20.60 ± 4.41	19.97 ± 3.72**	0.02	<0.00
	CS	16.61 ± 0.32	15.92 ± 0.44ns	24.35 ± 0.54	22.74 ± 0.45*	17.58 ± 3.66	16.89 ± 4.05**	0.04	<0.00
	W	16.78 ± 0.19	19.72 ± 0.49**	24.11 ± 0.38	23.94 ± 0.33ns	22.70 ± 3.33	19.82 ± 3.09**	0.13	0.01

* Significant at the $P=0.05$ level, ** Significant at the $P=0.01$ level

Histograms showed that the distribution followed a normal curve for all traits, indicating that the traits were additive in nature (Fig. 1). The water stress values, with lower values indicating lower levels of stress and higher values indicating more water stress, ranged from -1.05 to 0.70. Weslaco consistently had the lowest WS values with the exception of weight per pod where the value was 0.64 and the value of CC was 0.57. AVG DSIs are indicative of the amount of tolerance or susceptibility the population has; the lower the value, the more tolerant the population is to the stress for that trait. The lowest and highest AVG DSI among all average DSIs was found within the same trait, pods per plant, with CC having the lowest value of -0.09 and W having the highest at 2.83. Within all traits, the AVG DSIs were generally negatively correlated to the WS values, with the exception of PPP.

Broad sense heritability (H^2) estimates (Table 3) were obtained for all traits under both treatments. For the control plants, the broad sense heritability estimates ranged from 0.07 for PPP up to 0.94 for biomass. Heritability estimates for the drought treated entries ranged from 0 to 0.69. The estimates were lower for drought due to a larger proportion of the overall variance coming from environmental variance. Biomass and height under controlled treatments had the highest H^2 estimates.

Table 3 Heritability estimates for the IT98K-476-8 x GEC RIL population

Trait	Control					Drought				
	σ_g^2	σ_e^2	σ_{ge}^2	MSE	H^2	σ_g^2	σ_e^2	σ_{ge}^2	MSE	H^2
Days to flowering	38.37**	20.82**	46.89**	13.61	0.74	27.22**	6.85**	32.88**	27.09	0.69
Height	724.22**	43.74**	187.48**	47.46	0.87	82.65**	0.76*	19.68**	67.13	0.74
Biomass rating	3.85**	0.68**	0.09*	0.73	0.94	0.94**	2.29**	0.06ns	1.33	0.70
Pods per plant	0.78ns	7.72**	3.85ns	44.29	0.07	10.75*	77.56**	0.42ns	176.71	0.20
Total seed weight	21.46**	30.92**	19.56*	92.62	0.36	0.00ns	57.02**	1.09ns	150.73	0.00
Total pod weight	44.93**	56.80**	53.09**	151.63	0.38	0.00ns	33.44**	0.00ns	83.18	0.00
Weight per pod	0.10**	<0.00ns	0.03*	0.14	0.65	0.00ns	0.01ns	0.00ns	0.13	0.00
Hundred seed weight	6.30**	6.61**	10.10**	2.99	0.61	3.34**	2.83**	4.98**	7.23	0.48

* Significant at the $P=0.05$ level, ** Significant at the $P=0.01$ level

All traits, with the exception of height, biomass and pods per plant, had correlations with days to flowering at Weslaco; days to flower was not highly correlated to many traits in the other two locations. The vegetative traits HT and BM showed correlations in Corpus Christi (Table 4) and College Station (Table 5), but not in Weslaco (Table 6). Pods per plant had a negative correlation with DSI-PPP in CS. Total pod weight had strong correlations with total seed weight. One-hundred seed weight had positive correlations with weight per pod in CC and CS and pods per plant in CS. One-hundred seed weight in CC showed negative correlations with HT and BM.

Table 4 Correlations of phenotypic traits and DSIs in the RIL population at Corpus Christi

	DTF	HT	BM	TSW	PPP	TPW	WTPP	HSW	DSI-HT	DSI-BM	DSI-TSW	DSI-PPP	DSI-TPW	DSI-WTPP	DSI-HSW
DTF	- - -	0.03	0.08	-0.13	0.04	-0.12	-0.03	0.15	-0.15	-0.28**	-0.16	-0.03	-0.18*	-0.15	0.09
HT		- - -	0.77**	0.03	-0.01	0.02	0.04	0.08	0.52**	0.31**	0.05	0.03	0.01	0.04	0.03
BM			- - -	-0.02	-0.09	-0.03	0.06	0.13	0.56**	0.55**	0.07	-0.02	-0.01	-0.06	0.08
TSW				- - -	-0.02	0.99**	0.58**	0.15	-0.09	-0.22**	0.43**	0.26**	0.50**	0.55**	0.01
PPP					- - -	-0.01	0.02	-0.07	-0.03	-0.09	-0.09	-0.07	-0.18*	-0.03	-0.06
TPW						- - -	0.57**	0.08	-0.09	-0.23**	0.42**	0.26**	0.54**	0.54**	-0.01
WTPP							- - -	0.25**	-0.03	-0.10	0.28**	-0.07	0.47**	0.62**	0.04
HSW								- - -	-0.16	-0.11	-0.13	-0.13	0.05	0.18*	0.57**
DSI-HT									- - -	0.77**	0.48**	0.01	0.26*	-0.14	-0.08
DSI-BM										- - -	0.28*	-0.08	0.10	-0.20*	-0.10
DSI-TSW											- - -	0.81**	0.85**	0.07	-0.05
DSI-PPP												- - -	0.55**	-0.20*	0.18*
DSI-TPW													- - -	0.33**	0.01
DSI-WTPP														- - -	-0.07
DSI-HSW															- - -

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level

Table 5 Correlations of phenotypic traits and DSIs in the RIL population at College Station

	DTF	HT	BM	PPP	HSW	DSI-HT	DSI-BM	DSI-PPP	DSI-HSW
DTF	- - -	-0.01	0.07	-0.05	0.06	-0.25**	-0.15	0.17	0.11
HT		- - -	0.53**	-0.42**	-0.43**	0.64**	0.32**	0.36**	-0.26**
BM			- - -	0.06	-0.25**	0.45**	0.57**	0.11	-0.15
PPP				- - -	0.24**	-0.15	0.06	-0.59**	0.21*
HSW					- - -	-0.21**	-0.09	-0.31**	0.66**
DSI-HT						- - -	0.56**	0.03	-0.25**
DSI-BM							- - -	-0.09	-0.02
CS-PPP								- - -	-0.14
DSI-HSW									- - -

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level

Table 6 Correlations of phenotypic traits and DSIs in the RIL population at Weslaco

	DTF	HT	BM	TSW	PPP	TPW	WTPP	HSW	DSI-HT	DSI-BM	DSI-TSW	DSI-PPP	DSI-TPW	DSI-WTPP	DSI-HSW
DTF	- - -	-0.08	-0.01	-0.23**	0.09	-0.23**	0.16**	0.33**	-0.22**	-0.36**	0.23**	0.30**	-0.23**	0.23**	0.29**
HT		- - -	0.04	0.11	0.06	0.10	-0.05	-0.06	0.56**	0.03	0.15	0.10	-0.15	-0.12	-0.04
BM			- - -	-0.03	0.14	-0.04	0.01	0.06	-0.13	0.75**	0.08	0.06	-0.08	-0.07	0.09
TSW				- - -	-0.01	0.98**	0.30**	-0.08	0.15	-0.02	-0.23**	-0.28**	0.25**	0.21**	-0.10
PPP					- - -	-0.03	-0.02	0.11	-0.07	0.11	0.12	0.14	-0.13	0.15	0.03
TPW						- - -	0.32**	-0.07	0.12	-0.03	-0.22**	-0.28**	0.20**	0.22**	-0.07
WTPP							- - -	0.25**	-0.09	-0.17**	-0.16	-0.05	0.18**	0.70**	0.00
HSW								- - -	-0.18*	-0.12	0.08	0.11	-0.05	0.36**	0.45**
DSI-HT									- - -	0.06	0.02	-0.02	-0.03	-0.11	-0.01
DSI-BM										- - -	0.00	-0.04	0.00	-0.26**	-0.04
DSI-TSW											- - -	0.97**	-0.99**	0.01	-0.04
DSI-PPP												- - -	-0.97**	0.11	-0.05
DSI-TPW													- - -	0.02	0.02
DSI-WTPP														- - -	-0.01
DSI-HSW															- - -

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level

DSI-HT had positive correlations with height and biomass in CC, indicating that as a genotype's height and biomass increased, so did their percent reduction of height due to drought stress. DSI-HT was negatively correlated to days to flower and one-hundred seed weight in CS, indicating that as tolerance to reduction in height under stress was reduced, so was HSW and DTF. Though these correlations were

highly significant they were not strongly correlated. DSI-BM was negatively correlated to DTF, total seed weight and total pod weight in CC and to height and biomass in CC and CS.

2.3.2 Molecular mapping

Of the 180 genotypes that were genotyped, 175 had high quality allele calls. There was an average of 1,449,996 100-bp clean reads per genotype, with a mean genome coverage of 11.7x. Six-thousand and one SNPs were identified from the analysis of the RAD seq data, and from these, 4,154 SNPs were used in the construction of the linkage map. The constructed linkage map spanned 1,084.65 cM and covered 11 linkage groups, the haploid number of chromosomes for cowpea. The map has approximately one SNP marker every 0.26 cM or 149 kB. The markers were binned into 531 bins that did not co-segregate.

ICIM-Add mapping identified significant QTL at the permuted LOD thresholds for the traits days to flowering, height, biomass and DSI-BM. QTL were found at the LOD threshold of three for total seed weight, pods per plant, weight per pod and one-hundred seed weight. The QTL identified for DTF explained between 3.81 and 11.84 percent of the phenotypic variance (PVE) in the trait (Table 7). The twelve QTL for height explained from 2.31 to 6.67% of the phenotypic variance, and the 21 QTL mapped for biomass explained 1.03 and 2.66% of the variance in this trait (Table 8). All but one of the QTL for increased biomass were donated by IT98K476-8 while the majority of the height alleles were donated by GEC.

Table 7 Putative QTL identified in the IT98K-476-8 x GEC RIL population for days to flowering. QTL meeting the permuted LOD threshold are in bold, all others met the LOD threshold of three

Trait	LOD Threshold	LG	Position	Left Marker	Right Marker	Left CI[a]	Right CI	LOD	PVE[b]	Add[c]
DTF	7.29	1	19	10934	14537	13.5	20.5	3.03	3.81	0.51
		1	54	26418	6913	52.5	55.5	4.48	5.55	0.02
		2	8	306	285	5.5	9.5	5.71	7.44	0.04
		2	79	1761	717	77.5	83.5	3.15	4.09	0.18
		2	99	29178	20365	94.5	101.5	3.85	5.00	0.07
		2	117	10425	27998	115.5	117.5	3.56	4.59	-0.07
		2	137	21795	15125	133.5	139.5	6.04	7.79	-0.39
		2	157	3701	24570	155.5	159.5	3.41	4.54	-0.22
		3	18	8397	5650	12.5	21.5	4.94	5.99	-0.46
		4	29	14987	17477	25.5	30.5	3.16	4.03	0.51
		4	99	15538	27266	96.5	101.5	5.60	6.84	0.61
		4	139	8601	28522	134.5	142.0	7.15	8.95	0.02
		5	33	14621	24476	31.5	40.5	3.44	5.01	0.45
		5	54	13680	18342	48.5	57.5	3.13	4.12	0.45
		6	41	867	5862	34.5	54.5	4.65	6.63	-0.16
		7	9	6408	21863	4.5	11.5	3.52	3.85	0.32
		9	21	17017	29872	20.5	22.5	5.42	6.92	0.55
		10	**0**	**5711**	**19922**	**0.0**	**0.5**	**10.19**	**11.84**	**-0.80**
		10	9	19922	7535	5.5	11.5	6.43	9.64	-0.59
		10	30	1455	21737	27.5	32.5	4.39	5.87	-0.26
		10	62	33596	28820	61.5	64.5	3.25	4.34	0.14
		11	56	29040	23556	55.5	58.5	5.69	7.28	-0.83

[a] Left and right confidence intervals calculated by one LOD drop
[b] Percentage of variation explained by the QTL
[c] Additive effect of substituting the allele of IT98K-476-8 for an allele of GEC

Table 8 Putative QTL identified in the IT98K-476-8 x GEC RIL population for height and biomass. QTL meeting the permuted LOD threshold are in bold all others met the LOD threshold of three.

Trait	LOD Threshold	LG	Position	Left Marker	Right Marker	Left CI[a]	Right CI	LOD	PVE[b]	Add[c]
HT	5.47	1	52	28769	28369	46.5	56.5	4.16	3.79	4.91
		2	**32**	**4935**	**32313**	**29.5**	**33.5**	**5.60**	**6.67**	**-0.96**
		2	63	14254	12953	62.5	63.5	4.11	2.92	-4.90
		5	45	28103	27777	40.5	46.5	4.75	3.11	-4.54
		5	60	11570	17220	56.5	63.5	3.62	2.78	-4.29
		6	85	24063	19001	84.5	86.5	3.49	3.36	-1.90
		7	63	27269	30347	59.5	63.0	3.98	2.84	-3.12
		8	22	28767	11907	19.5	30.5	4.74	3.10	-4.33
		8	**71**	**2219**	**10165**	**65.5**	**72.5**	**8.84**	**5.74**	**-5.08**
		8	88	17047	2577	87.5	89.5	4.98	4.33	-2.64
		9	**81**	**7480**	**25931**	**78.5**	**82.0**	**5.92**	**4.59**	**-2.40**
		10	0	5711	19922	0.0	0.5	3.33	2.31	-2.86
BM	5.76	1	13	25559	7962	10.5	15.5	4.33	1.54	0.24
		1	24	17147	27414	22.5	26.5	3.02	1.03	0.19
		1	31	21050	19614	28.5	32.5	3.40	1.13	0.20
		1	51	26288	28769	45.5	51.5	3.22	1.23	0.22
		2	54	1311	682	50.5	56.5	4.13	1.74	0.38
		2	60	10939	14254	58.5	62.5	3.68	1.44	0.24
		2	95	21237	5898	90.5	98.5	5.74	2.34	0.29
		2	106	20365	4579	104.5	107.5	5.40	2.27	0.28
		2	**112**	**27515**	**25187**	**110.5**	**113.5**	**6.72**	**2.66**	**0.33**
		2	**128**	**7923**	**24629**	**126.5**	**130.5**	**6.69**	**2.52**	**0.33**
		2	**146**	**20714**	**25140**	**142.5**	**147.5**	**5.93**	**2.26**	**0.35**
		4	64	21402	26834	62.5	64.5	4.47	1.92	0.24
		4	**94**	**8586**	**26586**	**90.5**	**95.5**	**6.15**	**2.34**	**0.31**
		4	**99**	**15538**	**27266**	**97.5**	**99.5**	**6.05**	**2.26**	**0.31**
		5	**3**	**7053**	**25802**	**1.5**	**4.5**	**6.38**	**2.55**	**0.33**
		6	35	27501	867	33.5	44.5	3.06	1.09	0.20
		9	12	4882	6824	10.5	17.5	5.16	2.06	0.27
		9	24	10609	22736	23.5	28.5	4.94	1.96	0.27
		9	35	12300	5144	33.5	36.5	5.26	2.04	0.28
		10	**94**	**11487**	**30880**	**93.5**	**94**	**6.31**	**2.47**	**0.30**
		11	58	29040	23556	55.5	58.5	3.27	1.13	-0.21

[a] Left and right confidence intervals calculated by one LOD drop
[b] Percentage of variation explained by the QTL
[c] Additive effect of substituting the allele of IT98K-476-8 for an allele of GEC

The one QTL identified for total seed weight had a PVE of 54.61%, but did not reach the permuted threshold of 4.64 (Table 9). There were six QTL mapped for pods per plant that accounted for 7.91 to 11.52% of the variance. The three weight per pod QTL accounted for 2.52 to 2.64 percent of the data. One-hundred seed weight QTL accounted for 3.90 to 5.50 PVE.

Table 9 Putative QTL identified in the IT98K-476-8 x GEC RIL population for yield components total seed weight, pods per plant, weight per pod, and one-hundred seed weight. QTL meeting the permuted LOD threshold are in bold all others met the LOD threshold of three.

Trait	LOD Threshold	LG	Position	Left Marker	Right Marker	Left CI[a]	Right CI	LOD	PVE[b]	Add[c]
TSW	4.64	2	7	306	285	0.0	8.5	3.36	54.61	-0.21
PPP	5.82	2	2	306	285	0.0	9.5	3.68	9.16	-0.40
		2	117	10425	27998	115.5	118.5	3.04	7.91	-0.20
		4	140	12909	31080	139.5	140.5	3.10	8.44	-0.10
		9	36	5144	22102	33.5	39.5	3.29	8.24	-0.30
		9	78	7480	25931	75.5	82.0	3.17	11.52	-0.30
WTPP	4.67	3	95	16532	14976	92.5	100.5	3.05	2.64	0.06
		4	26	3064	14987	24.5	27.5	3.06	2.65	-0.07
		6	94	32261	26350	90.5	97.0	3.42	2.52	0.05
HSW	5.78	2	62	14254	12953	60.5	63.5	4.02	3.90	-0.36
		2	111	27667	27515	109.5	114.5	3.04	4.10	0.35
		3	43	3458	31330	40.5	48.5	3.45	4.80	-0.04
		6	66	8390	23002	65.5	66.5	3.40	4.00	-0.20
		8	62	1254	2219	55.5	69.5	3.12	4.60	-0.07
		8	85	20642	16585	79.5	86.5	3.85	5.50	-0.30
		9	14	20349	15133	12.5	20.5	3.25	4.00	0.33
		10	1	19922	7535	0.0	5.5	3.28	4.70	-0.37
		10	52	7003	30999	49.5	53.5	4.19	5.20	-0.16

[a] Left and right confidence intervals calculated by one LOD drop
[b] Percentage of variation explained by the QTL
[c] Additive effect of substituting the allele of IT98K-476-8 for an allele of GEC

Table 10 Putative QTL identified in the IT98K-476-8 x GEC RIL population for drought susceptibility indices. QTL meeting the permuted LOD threshold are in bold all others met the LOD threshold of three.

Trait	LOD Threshold	LG	Position	Left Marker	Right Marker	Left CI[a]	Right CI	LOD	PVE[b]	Add[c]
DSI-HT	4.05	2	49	3836	1311	46.5	50.5	3.37	14.70	0.02
DSI-BM	4.08	**2**	**50**	**3836**	**1311**	**48.5**	**50.5**	**5.02**	**4.68**	**0.22**
		2	131	24629	27188	126.5	134.5	3.13	5.13	0.15
		4	53	3568	4549	51.5	53.5	3.55	5.05	0.15
		5	**1**	**25761**	**7053**	**0.0**	**2.5**	**4.42**	**7.32**	**0.18**
DSI-TSW	2.97	**2**	**45**	**3836**	**1311**	**42.5**	**47.5**	**3.60**	**243.31**	**-0.01**

[a] Left and right confidence intervals calculated by one LOD drop
[b] Percentage of variation explained by the QTL
[c] Additive effect of substituting the allele of IT98K-476-8 for an allele of GEC

There was one QTL identified that was linked to DSI-HT (Table 10). This QTL explained

14.70% of the variance around this trait. The DSI-BM QTL explained between 4.68 and 7.32 percent of

the phenotypic variance. The QTL for DSI-TSW accounted for a whopping 243.31% of the phenotypic

variance as per the IciMapping software.

Table 11 Significant epistatic QTL for phenotypic traits and drought related indices

Trait	LOD Threshold	LG 1	Position 1	LG 2	Position 2	LOD	PVE[a]	PVE (AA)[b]	LOD (AA)[c]	Add by Add[d]
DTF	9.81	1	15	2	145	11.96	2.06	0.82	4.91	-0.86
		3	90	4	35	11.25	2.33	0.73	3.39	-0.75
		6	45	10	45	10.77	1.94	0.85	4.44	0.77
		8	45	10	50	11.87	2.56	1.79	8.52	-1.13
		7	0	10	90	9.99	1.98	0.13	0.59	-0.30
HT	9.45	3	40	3	45	20.96	1.68	1.68	17.31	-13.25
BM	7.89	2	0	2	15	15.69	4.98	4.98	15.58	-0.67
		1	0	2	125	12.86	4.52	4.47	12.69	-0.45
		1	60	3	85	8.00	3.07	2.96	7.73	0.36
		4	30	4	95	12.76	4.43	3.66	10.04	-0.46
		2	70	4	130	12.59	4.95	4.81	12.34	-0.53
		3	40	5	35	10.81	3.96	3.39	8.94	-0.40
		4	135	5	65	9.16	3.39	2.79	7.48	-0.36
		5	10	5	80	13.16	4.95	4.51	12.05	-0.47
		2	50	6	45	9.99	3.58	3.43	9.42	0.61
		2	0	7	30	13.11	4.83	4.63	12.52	-0.52
		5	60	8	80	12.04	4.30	3.81	10.37	-0.41
		7	55	8	95	9.81	3.52	3.24	8.86	-0.38
		4	65	8	105	8.34	3.05	2.99	8.17	-0.37
		2	160	8	110	10.92	4.15	3.95	10.35	0.43
		2	65	9	25	9.83	3.80	3.37	8.43	-0.44
		3	110	9	65	10.74	3.89	3.52	9.62	0.40
		5	0	9	75	8.30	2.82	2.37	6.79	0.35
		3	90	10	0	8.59	3.12	2.21	5.96	0.31
		4	75	10	10	8.38	3.06	2.44	6.74	0.34
		6	10	10	15	11.62	4.37	4.05	10.85	-0.42
		8	10	10	15	9.42	3.52	3.34	8.93	0.38
		1	35	10	50	9.43	3.44	3.26	8.92	0.38
		10	50	10	60	9.26	2.86	2.86	8.72	0.62
		9	50	10	85	10.17	3.76	3.60	9.78	-0.40
		4	125	11	0	8.97	3.44	3.29	8.53	0.39
		5	40	11	5	8.27	3.09	3.00	8.00	0.36
		9	25	11	5	10.20	3.70	3.53	9.70	0.40
		7	50	11	50	8.07	2.93	2.52	6.69	-0.33
PPP	10.35	2	150	2	155	10.65	0.09	0.09	0.82	-0.60
HSW	8.04	2	160	10	65	8.98	2.83	1.49	4.95	-0.49
		5	35	10	85	10.51	2.99	1.33	4.74	0.44
		4	10	11	5	8.52	2.53	2.45	8.10	-0.63
DSI-BM	17.75	2	40	2	45	40.91	2.70	2.70	22.76	0.63
		3	30	3	35	31.60	0.79	0.79	16.83	0.47

[a] Percentage of variation explained by the main effect QTL

[b] Percentage of variation explained by the epistatic interaction

[c] The effect of the additive by additive interaction between two loci; negative values indicate that the interaction was between alleles originating from different parents

ICIM-EPI mapping found QTL involved in epistatic interactions for the traits DTF, HT, BM, PPP, HSW and DSI-BM (Table 11). For DTF, five interactions met the LOD threshold and the main effect QTL explained up to 2.06% of the phenotypic variance. The one significant digenic interaction mapped for HT was negative indicating that the interaction was between loci that derived from different parents and was a result of recombination. There were 28 significant epistatic interactions between the LODs of eight and 15.69 mapped for BM and the interaction explained between 2.21% and 4.98% of the variance of this trait. For PPP, only one interaction was identified. This interaction's additive by additive interaction was negative, indicating that it was between recombinant loci, where a positive value would indicate that the epistasis occurred between loci from the same genotype. There were three pairs of epistatic QTL mapped for HSW. Two epistatic interactions were identified for DSI-BM, which had LODS of 31.60 and 40.91, and explained 0.79 and 2.70 of the percentage of variation, respectively.

The additive QTL for DSI-TSW, DSI-HT and the epistatic QTL for biomass and DSI-BM was linked to two markers, 3836 and 1311, on LG 2 at position 45 and 50, that were used in a means contrast. These markers were also linked to loci with significant effects on pods per plant in Corpus Christi, and in College Station and on DSI-BM in Weslaco and DSI-HSW in College Station (Table 12).

Table 12 Traits found significant by LSmeans contrast for markers linked to additive QTL identified for, DSI-HT, DSI-BM, DSI-TSW and epistatic QTL for BM and DSI-BM at LG 2 positions 45-50

Marker		3836		3836		1311		1311	
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
HT	CC	41.71	5.61	51.74	16.86	56.72	6.23	31.98	13.41
HT	CS	36.21	2.94	41.12	8.90	42.79	3.27	30.99	7.07
HT	W	75.60	8.57	95.36	22.93	71.74	8.42	110.37	21.36
BM	CC	5.26	0.34	5.46	1.03	5.68	0.38	4.31	0.82
BM	CS	4.86	0.30	6.23	0.91	5.64	0.34	4.85	0.73
BM	W	6.39	0.29	7.15	0.86	6.94	0.32	5.86	0.68
TSW	CC	10.80	0.91	5.91	2.69	7.17	0.97	10.40	2.16
TSW	W	16.44	1.80	14.23	5.31	15.79	1.97	18.70	4.22
PPP	CC	8.61	0.59	7.31	1.88	6.64	0.67	11.03	1.50**
PPP	CS	12.19	0.84	15.33	2.54	11.79	0.94	16.49	2.02*
PPP	W	11.48	1.04	7.20	3.48	11.91	1.23	8.50	2.53

Table 12 Continued

Marker		3836		3836		1311		1311	
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
TPW	CC	14.63	1.22	9.27	3.58	10.33	1.29	14.43	2.87
TPW	W	22.63	2.49	19.26	7.35	21.69	2.72	26.21	5.85
WTPP	CC	1.87	0.08	1.58	0.25	1.64	0.09	1.81	0.20
WTPP	W	1.82	0.07	1.79	0.20	1.78	0.07	1.80	0.16
HSW	CC	19.85	0.83	20.29	2.30	20.48	0.82	19.05	1.97
HSW	CS	18.40	0.54	18.89	1.59	17.73	0.58	19.62	1.32
HSW	W	22.34	0.53	23.75	1.50	23.03	0.55	22.31	1.25
DSI-HT	CC	-0.10	0.08	-0.16	0.23	-0.04	0.09	-0.24	0.18
DSI-HT	CS	0.21	0.01	0.22	0.03	0.23	0.01	0.19	0.03
DSI-HT	W	-0.11	0.05	-0.05	0.14	-0.04	0.05	0.04	0.12
DSI-BM	CC	0.64	0.16	0.93	0.48	0.96	0.18	0.37	0.38
DSI-BM	CS	0.10	0.03	0.21	0.09	0.19	0.03	0.06	0.07
DSI-BM	W	0.42	0.20	0.95	0.59	1.01	0.22	0.04	0.49*
DSI-TSW	CC	0.09	0.05	-0.07	0.16	-0.02	0.06	0.01	0.13
DSI-TSW	W	0.10	0.14	-0.03	0.46	0.06	0.17	0.00	0.34
DSI-PPP	CC	-0.06	0.04	-0.12	0.12	-0.07	0.04	-0.13	0.10
DSI-PPP	CS	-0.02	0.07	-0.18	0.26	0.00	0.09	-0.16	0.18
DSI-PPP	W	2.45	1.24	0.90	3.98	2.08	1.48	1.35	2.91
DSI-TPW	CC	0.34	0.08	0.04	0.23	0.11	0.08	0.25	0.18
DSI-TPW	W	0.01	0.20	0.18	0.64	0.05	0.24	0.17	0.47
DSI-WTPP	CC	0.31	0.02	0.29	0.07	0.27	0.03	0.36	0.06
DSI-WTPP	W	1.29	0.92	0.32	2.94	1.08	1.09	0.47	2.14
DSI-HSW	CC	-0.0012	0.0009	-0.0013	0.0025	-0.0001	0.0009	-0.0026	0.0021
DSI-HSW	CS	0.0032	0.0023	-0.0100	0.0100*	-0.0048	0.0028	-0.0014	0.0056
DSI-HSW	W	0.0163	0.0025	0.0086	0.0089	0.0111	0.0032	0.0161	0.0061

* Significant at the P=0.05 level, ** Significant at the P=0.01 level
[a] Allele donated by IT98K-476-8
[b] Allele donated by Golden Eye Cream

Table 13 LSmeans contrast for markers linked to the additive QTL identified for days to flowering at LG 10 position 0

Marker		5711		5711		19922		19922	
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
HT	CC	23.64	16.94	60.65	11.36	58.63	14.30	26.01	19.02
HT	CS	25.42	8.86	42.31	5.94	51.97	7.48	37.92	9.95
HT	W	57.36	25.86	65.14	19.41	80.32	14.99	65.98	15.32
BM	CC	3.83	1.03	5.69	0.69	6.15	0.87	4.91	1.16
BM	CS	3.02	0.90	5.39	0.61*	6.10	0.76	3.95	1.01
BM	W	6.01	0.87	6.30	0.58	6.87	0.73	6.86	0.97
TSW	CC	13.44	2.73	13.68	2.04	8.59	2.05	9.40	2.72

Table 13 Continued

Marker		5711		5711		19922		19922	
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
TSW	W	24.66	5.93	15.53	4.45	9.10	4.45	19.14	5.92
PPP	CC	6.28	1.73	6.93	1.16	6.11	1.46	5.58	1.94
PPP	CS	6.93	2.55	12.44	1.71	11.99	2.15	5.50*	2.87
PPP	W	14.45	3.15	11.00	2.11	10.62	2.04	14.41	2.09
TPW	CC	18.20	3.62	18.25	2.71	11.81	2.72	13.33	3.61
TPW	W	34.52	8.24	22.64	6.19	13.62	6.19	26.38	8.23
WTPP	CC	1.74	0.25	1.77	0.18	1.55	0.19	1.69	0.25
WTPP	W	1.74	0.22	1.69	0.17	1.76	0.17	1.96	0.22
HSW	CC	23.19	2.25	19.58	1.69	17.79	1.69	22.68	2.25
HSW	CS	19.72	1.57	17.48	1.05	16.46	1.33	19.49	1.76
HSW	W	22.67	1.67	22.57	1.26	22.14	1.26	23.00	1.67
DSI-HT	CC	-0.30	0.27	0.02	0.18	0.05	0.16	-0.21	0.17
DSI-HT	CS	0.20	0.04	0.21	0.03	0.22	0.03	0.22	0.03
DSI-HT	W	-0.04	0.14	-0.08	0.09	-0.05	0.09	-0.08	0.09
DSI-BM	CC	0.64	0.48	0.71	0.32	1.04	0.40	1.02	0.54
DSI-BM	CS	-0.01	0.11	0.13	0.07	0.27	0.07	0.13	0.11
DSI-BM	W	0.43	0.59	0.27	0.40	0.93	0.50	1.09	0.67
DSI-TSW	CC	-0.02	0.18	0.10	0.13	0.11	0.13	-0.05	0.18
DSI-TSW	W	0.06	0.46	0.09	0.35	0.14	0.35	0.20	0.46
DSI-PPP	CC	-0.10	0.14	-0.01	0.10	0.01	0.10	-0.14	0.14
DSI-PPP	CS	-0.04	0.33	0.04	0.21	-0.02	0.21	0.01	0.33
DSI-PPP	W	1.46	4.02	1.90	3.01	2.69	3.02	3.02	4.01
DSI-TPW	CC	0.28	0.26	0.27	0.18	0.31	0.18	0.19	0.26
DSI-TPW	W	0.09	0.65	0.02	0.48	-0.06	0.49	-0.09	0.65
DSI-WTPP	CC	0.29	0.08	0.33	0.06	0.29	0.06	0.28	0.08
DSI-WTPP	W	0.48	2.97	0.79	2.23	0.48	2.23	1.15	2.97
DSI-HSW	CC	0.0032	0.0028	-0.0019	0.0020	-0.0033	0.0020	0.0027	0.0028
DSI-HSW	CS	0.0033	0.0100	-0.0002	0.0064	0.0015	0.0064	0.0066	0.0100
DSI-HSW	W	0.0098	0.0077	0.0153	0.0058	0.0176	0.0058	0.0162	0.0077

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level
[a] Allele donated by IT98K-476-8
[b] Allele donated by Golden Eye Cream

The markers found significant for days to flower was found to also be significant for pods per plant in College Station (Table 13). The markers linked to height on LG 8 position 71 were also found to be significant for biomass, total seed weight, total pod weight, DSI-WTPP and DSI-HSW (Table 14).

Table 14 LSmeans contrast for markers linked to the additive QTL identified for height at LG 8 position 71

Marker		2219				10165			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
HT	CC	48.41	5.12	41.65	5.54	39.30	7.52	50.71	5.54
HT	CS	38.63	2.63	35.29	2.86	33.36	3.92	39.54	2.81
HT	W	80.04	7.24	67.85	7.91	61.18	10.56	77.06	7.63
BM	CC	5.43	0.31	4.80	0.34	4.52	0.46	5.84	0.34*
BM	CS	5.39	0.27	4.73	0.30	4.80	0.40	5.51	0.29
BM	W	6.53	0.26	6.13	0.28	6.22	0.38	6.76	0.28
TSW	CC	8.12	0.81	10.63	0.85*	10.87	1.18	8.42	0.88
TSW	W	16.95	1.67	16.38	1.79	15.90	2.51	16.38	1.79
PPP	CC	7.09	0.54	6.90	0.59	6.91	0.80	7.27	0.58
PPP	CS	12.29	0.77	10.61	0.85	11.18	1.18	12.22	0.83
PPP	W	12.67	0.94	10.62	1.01	11.22	1.39	12.98	0.98
TPW	CC	11.20	1.07	14.83	1.13*	14.99	1.56	11.43	1.16
TPW	W	23.43	2.32	22.84	2.49	21.67	3.48	22.91	2.48
WTPP	CC	1.72	0.07	1.86	0.08	1.89	0.11	1.68	0.08
WTPP	W	1.87	0.06	1.82	0.07	1.83	0.09	1.78	0.07
HSW	CC	20.22	0.67	21.01	0.71	21.38	0.99	20.52	0.72
HSW	CS	18.44	0.48	17.49	0.52	17.40	0.71	17.89	0.50
HSW	W	22.67	0.47	22.88	0.50	22.43	0.71	22.82	0.49
DSI-HT	CC	0.00	0.08	-0.21	0.08	-0.21	0.11	0.06	0.08
DSI-HT	CS	0.22	0.01	0.22	0.01	0.21	0.01	0.22	0.01
DSI-HT	W	-0.06	0.04	-0.07	0.05	-0.14	0.06	-0.08	0.05
DSI-BM	CC	0.84	0.15	0.54	0.16	0.44	0.22	0.96	0.16
DSI-BM	CS	0.14	0.03	0.13	0.03	0.15	0.04	0.16	0.03
DSI-BM	W	0.73	0.19	0.52	0.20	0.64	0.27	0.85	0.20
DSI-TSW	CC	0.05	0.05	-0.05	0.05	0.02	0.07	0.07	0.05
DSI-TSW	W	0.28	0.13	0.03	0.14	0.07	0.20	0.15	0.15
DSI-PPP	CC	-0.09	0.04	-0.13	0.04	-0.10	0.05	-0.07	0.04
DSI-PPP	CS	0.00	0.07	0.06	0.07	0.02	0.10	0.03	0.07
DSI-PPP	W	4.03	1.14	1.82	1.24	2.98	1.70	2.67	1.27
DSI-TPW	CC	0.25	0.07	0.13	0.07	0.22	0.10	0.25	0.07
DSI-TPW	W	-0.23	0.18	0.11	0.20	0.02	0.27	-0.06	0.20
DSI-WTPP	CC	0.26	0.02	0.36	0.02**	0.36	0.03	0.25	0.02**
DSI-WTPP	W	0.73	0.86	1.26	0.92	0.53	1.26	1.44	0.94
DSI-HSW	CS	0.0050	0.0021*	-0.0021	0.0023	0.0012	0.0031	0.0002	0.0022
DSI-HSW	W	0.0131	0.0022	0.0134	0.0024	0.0095	0.0033	0.0182	0.0024
DSI-HSW	CC	-0.0008	0.0008	0.0002	0.0008	0.0012	0.0011	-0.0003	0.0008

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level
[a] Allele donated by IT98K-476-8
[b] Allele donated by Golden Eye Cream

Table 15 LSmeans contrast for markers linked to the additive QTL identified for total seed weight at LG 2 position 7

Marker		306				285			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
HT	CC	54.94	7.92	57.15	6.66	51.70	12.37	47.99	4.03
HT	CS	43.08	4.17	40.71	3.46	37.69	6.51	40.06	2.11
HT	W	78.03	11.47	67.26	9.49	78.27	17.92	77.29	5.79
BM	CC	5.83	0.49	5.62	0.41	5.73	0.77	5.34	0.25
BM	CS	4.69	0.42	5.33	0.36	4.75	0.67	5.12	0.22
BM	W	6.94	0.41	6.69	0.34	6.84	0.64	6.55	0.21
TSW	CC	10.54	1.48	9.57	1.10	11.10	2.25	9.74	0.66
TSW	W	16.19	2.67	15.11	2.18	15.91	4.16	17.41	1.32
PPP	CC	7.82	0.82	7.10	0.67	8.99	1.26	6.95	0.41
PPP	CS	9.08	1.18	10.43	0.98	9.88	1.84	11.02	0.60
PPP	W	16.30	1.48	12.75	1.22	15.09	2.42	12.82	0.73
TPW	CC	14.45	1.97	13.60	1.48	15.25	2.99	13.30	0.87
TPW	W	24.57	3.69	22.15	3.02	26.22	5.75	24.23	1.82
WTPP	CC	1.73	0.13	1.85	0.10	1.87	0.20	1.76	0.06
WTPP	W	1.91	0.10	1.79	0.08	1.92	0.16	1.85	0.05
HSW	CC	20.07	1.20	18.78	0.88	17.87	1.82	21.23	0.54
HSW	CS	16.45	0.73	16.75	0.61	16.79	1.14	17.97	0.37
HSW	W	23.13	0.75	22.28	0.61	22.19	1.17	23.06	0.37
DSI-HT	CC	-0.04	0.12	0.06	0.10	0.05	0.20	-0.14	0.06
DSI-HT	CS	0.22	0.01	0.22	0.01	0.22	0.02	0.22	0.01
DSI-HT	W	0.00	0.07	-0.15	0.06	-0.08	0.10	-0.03	0.04
DSI-BM	CC	0.66	0.23	0.62	0.20	0.38	0.35	0.81	0.12
DSI-BM	CS	0.06	0.05	0.09	0.04	-0.06	0.08	0.16	0.02
DSI-BM	W	0.83	0.30	0.65	0.25	0.50	0.47	0.77	0.15
DSI-TSW	CC	0.01	0.08	0.04	0.06	0.11	0.13	0.00	0.04
DSI-TSW	W	0.08	0.21	0.31	0.17	0.28	0.32	0.11	0.11
DSI-PPP	CS	0.24	0.10	-0.02	0.09*	-0.08	0.17	0.07	0.05
DSI-PPP	W	2.37	1.87	4.36	1.55	4.02	2.97	2.26	0.92
DSI-PPP	CC	-0.06	0.06	-0.06	0.05	0.00	0.10	-0.10	0.03
DSI-TPW	CC	0.13	0.12	0.23	0.09	0.33	0.19	0.20	0.06
DSI-TPW	W	0.06	0.30	-0.29	0.24	-0.18	0.45	0.00	0.15
DSI-WTPP	CC	0.32	0.04	0.29	0.03	0.33	0.06	0.32	0.02
DSI-WTPP	W	5.19	1.22	3.00	1.05	8.96	1.91	0.94	0.63**
DSI-HSW	CS	-0.001	0.003	-0.002	0.003	-0.001	0.006	0.001	0.002
DSI-HSW	W	0.019	0.004	0.016	0.003	0.024	0.005	0.015	0.002
DSI-HSW	CC	0.000	0.001	-0.001	0.001	-0.002	0.002	0.000	0.001

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level
[a] Allele donated by IT98K-476-8
[b] Allele donated by Golden Eye Cream

The markers on LG 2 at position 7 that were associated with total seed weight were also significant for pods per plant, DSI-BM, DSI-PPP and DSI-WTPP (Table 15). The epistatic QTL pair that was significant for height were both used in contrasts, but only one marker of the four analyzed had a significant effect on the least squared mean (Tables 16 & 17).

Table 16 LSmeans contrast for markers linked to the epistatic QTL identified for height at LG 3 position 40

Marker		3458				31330			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
HT	CC	47.62	6.91	44.45	6.10	46.57	6.68	52.45	4.75
HT	CS	37.22	3.62	35.82	3.17	37.53	3.50	40.56	2.46
HT	W	85.28	9.78	80.53	8.41	81.99	9.12	74.25	6.60
BM	CC	5.28	0.42	4.96	0.37	5.44	0.41	5.55	0.29
BM	CS	5.30	0.37	4.96	0.32	4.85	0.37	5.72	0.25
BM	W	6.42	0.35	6.26	0.31	6.54	0.34	6.71	0.24
TSW	CC	11.11	1.08	8.96	0.97	10.00	1.03	9.81	0.75
TSW	W	17.48	2.24	15.54	1.97	15.72	2.13	16.39	1.51
PPP	CC	8.10	0.73	6.34	0.65	7.85	0.73	7.12	0.48
PPP	CS	10.65	1.07	11.38	0.93	11.15	1.03	11.86	0.72
PPP	W	13.27	1.29	11.62	1.09	10.95	1.25	13.85	0.85
TPW	CC	15.17	1.43	12.35	1.29	13.75	1.37	13.73	1.00
TPW	W	23.45	3.11	21.45	2.74	22.64	2.95	22.24	2.10
WTPP	CC	1.77	0.10	1.73	0.09	1.84	0.09	1.73	0.07
WTPP	W	1.82	0.08	1.78	0.07	1.85	0.08	1.79	0.06
HSW	CC	21.34	0.90	21.09	0.79	21.00	0.87	21.38	0.62
HSW	CS	18.41	0.64	17.31	0.57	18.57	0.62	17.27	0.44
HSW	W	22.48	0.63	22.96	0.55	22.82	0.61	23.16	0.42
DSI-HT	CC	-0.06	0.11	-0.19	0.09	-0.02	0.10	-0.06	0.07
DSI-HT	CS	0.22	0.01	0.21	0.01	0.22	0.01	0.22	0.01
DSI-HT	W	0.03	0.06	-0.04	0.05	-0.04	0.06	-0.08	0.04
DSI-BM	CC	0.59	0.19	0.60	0.17	0.90	0.18	0.79	0.13
DSI-BM	CS	0.12	0.04	0.15	0.03	0.13	0.04	0.16	0.03
DSI-BM	W	0.63	0.24	0.52	0.21	0.64	0.23	0.91	0.17
DSI-TSW	CC	0.04	0.06	-0.08	0.06	0.08	0.06	-0.01	0.13
DSI-TSW	W	0.17	0.18	0.32	0.16	0.24	0.17	0.23	0.04
DSI-PPP	CS	0.03	0.09	0.17	0.08	0.00	0.08	0.04	0.06
DSI-PPP	W	2.74	1.56	4.10	1.36	4.08	1.46	3.12	1.10
DSI-PPP	CC	-0.04	0.05	-0.14	0.04	-0.02	0.05	-0.10	0.03
DSI-TPW	CC	0.23	0.09	0.16	0.08	0.30	0.09	0.14	0.06
DSI-TPW	W	-0.10	0.25	-0.28	0.22	-0.22	0.23	-0.16	0.18

Table 16 Continued

Marker		3458				31330			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
DSI-WTPP	CC	0.31	0.03	0.31	0.03	0.31	0.03	0.30	0.81
DSI-WTPP	W	3.00	1.12	0.32	0.99	0.32	1.07	1.13	0.00
DSI-HSW	CS	0.001	0.003	-0.002	0.002	0.002	0.003	-0.002	0.002
DSI-HSW	W	0.013	0.003	0.013	0.003	0.017	0.003	0.015	0.001
DSI-HSW	CC	0.001	0.001	0.000	0.001	0.000	0.001	0.001	0.021

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level
[a] Allele donated by IT98K-476-8
[b] Allele donated by Golden Eye Cream

Table 17 LSmeans contrast for markers linked to the epistatic QTL identified for height at LG 3 position 45

Marker		31330				334			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
HT	CC	48.52	6.64	51.27	5.07	43.45	8.16	47.94	4.04
HT	CS	38.65	3.49	40.28	2.64	36.24	4.29	38.83	2.12
HT	W	78.76	9.05	76.91	7.03	94.20	11.47	73.60	5.56
BM	CC	5.60	0.40	5.43	0.31	4.91	0.50	5.39	0.25
BM	CS	4.89	0.36	5.58	0.27	4.88	0.44	5.24	0.22
BM	W	6.66	0.34	6.59	0.26	6.06	0.41	6.55	0.20
TSW	CC	10.14	1.04	9.44	0.81	9.85	1.26	9.41	0.64
TSW	W	15.79	2.10	17.19	1.59	19.74	2.53	16.29	1.29
PPP	CC	8.17	0.72	7.14	0.52	8.12	0.86	7.11	0.43
PPP	CS	11.21	1.02	11.85	0.77	10.67	1.24	11.49	0.62
PPP	W	11.32	1.24	13.84	0.90	13.02	1.46	11.54	0.74
TPW	CC	13.85	1.37	13.19	1.08	13.46	1.66	13.21	0.85
TPW	W	22.67	2.92	23.38	2.21	26.76	3.52	23.07	1.79
WTPP	CC	1.85	0.09	1.73	0.07	1.76	0.11	1.79	0.06
WTPP	W	1.88	0.08	1.78	0.06	1.77	0.10	1.81	0.05
HSW	CC	21.05	0.87	21.75	0.66	22.30	1.04	20.16	0.52
HSW	CS	18.60	0.62	17.22	0.47	18.32	0.77	17.96	0.38
HSW	W	23.00	0.60	23.58	0.44	23.64	0.72	22.29	0.36
DSI-HT	CC	0.03	0.10	-0.08	0.08	-0.12	0.12	-0.08	0.06
DSI-HT	CS	0.23	0.01	0.22	0.01	0.22	0.02	0.22	0.01
DSI-HT	W	-0.05	0.06	-0.08	0.04	0.02	0.07	-0.08	0.03

Table 17 Continued

Marker		31330				334			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
DSI-BM	CC	1.01	0.18	0.89	0.14	0.88	0.23	0.69	0.11
DSI-BM	CS	0.14	0.04	0.17	0.03	0.15	0.05	0.14	0.02
DSI-BM	W	0.75	0.23	0.86	0.18	0.47	0.28	0.69	0.14
DSI-TSW	CC	0.12	0.06	0.02	0.05	0.07	0.07	-0.01	0.04
DSI-TSW	W	0.18	0.17	0.23	0.14	0.16	0.21	0.19	0.10
DSI-PPP	CC	0.00	0.05	-0.10	0.04	-0.06	0.05	-0.10	0.03
DSI-PPP	CS	-0.03	0.08	0.02	0.07	-0.03	0.10	-0.01	0.05
DSI-PPP	W	3.75	1.46	2.99	1.18	2.37	1.82	3.11	0.91
DSI-TPW	CC	0.35	0.09	0.17	0.07	0.30	0.10	0.18	0.05
DSI-TPW	W	-0.13	0.23	-0.15	0.19	-0.05	0.29	-0.14	0.14
DSI-WTPP	CC	0.31	0.03	0.30	0.02	0.32	0.03	0.30	0.02
DSI-WTPP	W	0.28	1.08	0.18	0.88	0.23	1.34	1.19	0.67
DSI-HSW	CC	0.0003	0.0010	0.0007	0.0007	0.0012	0.0012	-0.0001	0.0006
DSI-HSW	CS	0.0025	0.0026	-0.0029	0.0021	-0.0002	0.0033	0.0013	0.0017
DSI-HSW	W	0.0183	0.0028	0.0138	0.0023	0.0110	0.0036	0.0145	0.0018

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level
[a] Allele donated by IT98K-476-8
[b] Allele donated by Golden Eye Cream

2.4 Discussion

The environments in which these experiments were performed were not uniform in drought stress which makes the comparisons of these genotypes under drought more difficult. Due to the normal periods of low precipitation during the summers in Texas, drought stress was achieved at College Station location in 2014, but due to the effects of increased precipitation during August in 2015 (Table 18), due to El Nino, Weslaco and Corpus Christi only exhibited light and moderate stress, respectively. This is evident from the water stress value obtained from comparing the stress levels of the control and drought treatments as well as trait correlations between the two treatments in these locations.

Table 18 Monthly precipitation (precip) (cM) and mean monthly temperature (MT) (˚C) in experimental locations and years

Month	CC 2015		CS 2014		W 2015	
	Precip	MT	Precip	MT	Precip	MT
May	21.69	25.61	22.89	23.06	10.01	27.06
June	5.05	27.00	4.09	29.00	4.83	28.97
July	2.03	28.06	3.63	29.44	2.54	29.87
Aug	8.41	28.72	1.98	30.72	15.09	30.54
Sept	5.18	27.92	16.69	27.53	9.86	11.28

DTF was significantly different among the genotypes, and it was suspected that the identification of significant QTL conferring drought susceptibility would be difficult to unsuccessful due to the confounding effect of this trait on others. Pearson's correlations of phenotypic traits and drought indices to DTF showed that this was not always the case. Correlations with DTF were seen for most yield traits and drought susceptibility indices in Weslaco, but were not observed for the other locations, indicating that the phenotypic traits and drought regulating loci are not necessarily confounded by flowering and flowering loci; it is also possible that this was further affected by some level of photoperiod differences between environments and photoperiod sensitivity between genotypes. Days to flowering showed no significant change in all environments between irrigation treatments except for the most stressed environment, where both of the parental genotypes expressed an escape response by decreasing DTF. The average of the RIL population on the other hand had an increased DTF. This is possibly due to the additive nature of maturity or the influence of some photoperiod sensitivity loci that are not expressed in the parental lines. Of the additive DTF QTL mapped, four included loci residing on LG 10 (Fig.2) with large LOD scores, and many of the epistatic interaction were also involved with LG 10, indicating that LG 10 is a region associated with this trait. DTF is highly heritable, therefore use of markers linked to genes with large effects should be possible.

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Position	Marker	
0.00	5711	**DTF**
0.89	19922	
		DTF
9.44	7535	
14.84	19388	
17.21	4880	
17.53	28408	
20.32	33244	
26.60	22939	**DTF**
29.18	1455	
32.83	21737	
33.12	30174	
37.57	33432	
38.16	26325	
40.96	24689	
41.60	21480	
42.71	26097	
43.40	3899	
44.00	31350	
48.59	33173	*DTF*
48.89	23683	*DTF*
50.18	7003	
52.51	30999	
53.13	8306	
53.72	20000	
55.81	24255	
57.82	7098	
59.06	26915	
59.40	9851	
59.91	19825	
60.91	30084	
61.22	4106	
61.80	33596	**DTF**
63.40	28820	
63.71	22586	
67.49	22523	
67.80	7386	
68.44	27401	
68.75	11038	
69.07	3751	
69.37	12892	
71.06	15560	
72.18	17785	
76.92	12924	
77.54	33452	
78.49	27790	
86.09	30572	
86.48	12473	
89.34	8544	
90.70	11487	*DTF*
94.82	30880	

Fig. 2 LG 10 on the cowpea linkage map showing days to flowering QTL. Additive QTL are in bold, with the QTL that meet the permuted threshold underlined and the QTL involved in epistatic interactions are italicized.

Plant height and biomass also differed significantly among the parental genotypes, which unlike DTF, did not pose an issue for QTL mapping. HT and BM traits were highly correlated since plants that have the longest main stem generally had the largest amount of vegetative matter. Therefore many of the conclusions that can be made about one trait can be made about the other. There were more QTL mapped for BM and DSI-BM than HT and DSI-HT though, most likely due to phenotyping error of height. The largest values of HT and BM were recorded for IT98K-476-8, and therefore it made sense that all of the mapped QTL at the permutation threshold for BM and HT were mapped due to the effects of alleles donated by IT98K-476-8. Many of the QTL that were identified at LOD threshold of three for height had alleles donated by GEC, which was in contrast to the QTL mapped for biomass. This could be explained by the large affect epistatic QTL for height possibly interfering with the expression of this trait in GEC.

Height and biomass were positively correlated with the DSIs of the traits, indicating that as BM and HT increased, their susceptibility to reduction increased under drought. This was evidenced by larger percent reductions under drought treatment in HT for IT98K-476-8 in Corpus Christi and Weslaco and for BM in College Station and Weslaco. The fact that two of the QTLs involved in epistatic interactions for BM and DSI-BM and one additive QTL for DSI-BM all mapped to the same position, LG 2 position 50, further supported the hypothesis that with greater BM and HT, came greater susceptibility to drought for that trait when tolerance was defined as the ability to maintain yield under stress. If, after the reduction of BM due to drought stress, the plant had a larger amount of BM relative to other genotypes, it may also be considered drought tolerant where tolerance is defined as the ability to outcompete genotypes under drought stress.

Because Weslaco was planted in late June, the onset of precipitation at this location coincided with flowering with some of the later maturing lines and pod filling of the earlier ones. This timely precipitation most likely alleviated the stress during the physiological time periods in which drought stress would be most detrimental; flowering and pod filling. Stress during flowering can affect reproductive ability and therefore pods will not set or the number of seeds per pod will be diminished (Turk et al. 1980). Water stress during pod set is known to decrease seed weight (Turk et al. 1980), which will affect the HSW and TSW. The below average temperatures for this location also likely reduced drought stress due to reduced evaporation and transpiration.

Total seed weight at Weslaco increased for all genotypes and RILs due to the timely precipitation. In Corpus Christi, where more stress was induced, TSW was greatly reduced in IT98K-476-8, but was less so for GEC. Total seed weight was correlated with many yield related traits, which was to be expected. Total seed weight had a weak, but highly significant negative correlation to DSI-BM, indicating that as TSW increased, susceptibility to reductions in biomass decreased. This correlation suggests that with ability to maintain BM under stress, yield also increased. TSW was also positively correlated with DSI-TSW, DSI-TPW, DSI-PPP and DSI-WTPP which indicated as a genotype's potential for yield increased, so did the potential for drought susceptibility to yield. There was one QTL mapped for

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total seed weight. This QTL explained a large percentage of the variance of the trait, but did not have a large additive effect.

Decreasing pods per plant is believed to be a mechanism of tolerance under drought stress (Pandey et al. 1984; Turk et al. 1980), and that was observed in the most stressful environment, College Station, for the parents and the RIL population. Though the water stress value at CS was 0.22, the average DSI was -0.09 indicating that some RILs increased the number of pods under stress. The handful of QTL at LOD three for this trait all had negative additive values which reflected the donation of alleles from the parent GEC which usually had the largest number of pods per plant. There was one pair of epistatic QTL mapped for PPP involving loci associated with markers that were next to each other on the linkage map. The additive by additive value of this interaction was negative, indicating that the epistatic interaction took place among alleles from different parents (Table #).

Total pod weight was reduced in Corpus Christi where there were no timely rains, unlike Weslaco, where the timely rains allowed for increased pods per plant and total pod weight. Total pod weight had a negative correlation with DSI-BM indicating that as the tolerance to drought stress for biomass increased, so did the total pod weight. In Corpus Christi, the correlation of this trait was like that of total seed weight, with positive values for DSI-TSW, DSI-PPP, DSI-TPW and DSI-WTPP. This indicates that as potential for TPW increases, so did the susceptibility to drought for these traits.

One-hundred seed weight had a slight, but highly significant decrease in all locations among the parents and RILs with exception of IT98K-476-8 in Weslaco. GEC showed slight reductions in HSW in all environments. Even though HSW was reduced for GEC for this trait, weight per pod was significantly increased in this genotype. This effect could only be credited to an increased number of seeds per pod, which was not recorded in this study. One-hundred seed weight had a small positive correlation with DSI-WTPP and a moderate positive correlation with DSI-HSW. The correlation of HSW to DSI-WTPP could be attributed to a decrease in number of seeds per pod under drought stress. The correlation with DSI-HSW could be explained in the same manner as some of the other correlations with the DSIs, where the larger the genetic potential for a trait, the more susceptible that trait was to drought. Pearson's correlations showed a negative correlation of HSW to BM. The co-localization of QTL involved in epistatic

interactions with epistatic BM QTL at four different loci, LG 2 position 160, LG 5 position 35, LG 10 position 85 and LG 11 position 5, further supported this correlation. The additive values of the QTL were both positive and negative indicating that both parents had an influence on this trait.

From this study, it became more evident how the two parental genotypes differed in tolerance mechanisms. The data indicated that GEC decreases input into individual seeds in order to maintain seed number, thus maintaining its total seed weight and reducing its one-hundred seed weight. IT98K-476-8, on the other hand, invests in viable seed as evidenced by the increase in one-hundred seed weight and decrease in total seed weight under stress. It is possible that this shift in carbohydrate diversion under stress was not due to a genetically triggered response, but due to reduced fertility of IT98K-476-8. In working with these genotypes, it has been noted that GEC contains high levels of pollen and is very easy to use as a male parent. On the other hand, IT98K-476-8 has less pollen and crosses involving this genotype as the male parent will be successful approximately one out of 100 times. Pollen number reduction and viability under water stress has been studied for various crops and has been found to reduce yields (Fang et al. 2010) (Rang et al. 2011) (Ravikumar et al. 2003). This effect, coupled with the fact that IT98K-476-8 already has a small amount of pollen, likely reduced yields under stress.

Another trait worth noting is the weight of the carpel, or pod itself. Many of the pods exhibited thinner walls under drought and this is reflected in the value obtained when total seed weight is subtracted from total pod weight, especially for the RIL population. This may be a mechanism in which the plant changes its sink in order to ensure viable seed.

In all, 13 additive QTL and 40 epistatic QTL interactions at permuted LOD thresholds were mapped to the cowpea genome. For some of the traits the permuted LOD threshold was higher than the generally accepted LOD threshold of three. This may have been due to high marker density coupled with a small genome size (~660Mb) or the distribution of phenotypes in the population. The majority of QTL that were mapped at the higher threshold were for traits or indices that the parents differed dramatically in and were phenotyped in more than two locations; DTF, HT, BM, PPP, HSW and DSI-BM. It is evident from this that the third (and fourth, in the case of DTF) location, gave the mapping more power to discern QTL.

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The ANOVA proved that the RIL population did differ significantly for drought susceptibility, but despite this, mapping of the DSIs did not return any significant QTL besides DSI-BM at the permuted LOD threshold. This may be due to the susceptibility being so increased that yields could not be obtained. When yields were not obtained, it was reported as missing data and it was most likely this missing data that most hindered mapping efforts. Another explanation is that these two parental genotypes of cowpea are both tolerant to drought though they both behave differently in water stressed situations, and therefore the data collected for the yield traits may have been too similar to for successful QTL mapping.

Days to flowering QTL were spread across the genome though and have been found to co-localize with BM at three different loci. This co-localization is to be expected since BM accumulation slows after the initiation of flowering (Scully and Wallace 1990). The major QTL for day to flowering was located on LG 10 (Fig. 3) and many of the epistatic QTL for this trait involve loci that are also located on this LG, indicating that LG 10 is a hotspot for DTF regulation differences between the two parents of this population.
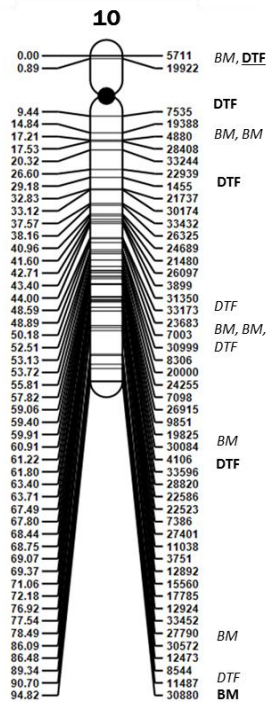
Fig. 3 LG 10 on the cowpea linkage map showing days to flowering and biomass QTL. Additive QTL are in bold, with the QTL that meet the permuted threshold underlined and the QTL involved in epistatic interactions are italicized.

The QTL for the vegetative and yield traits were also spread across the genome, but many resided on LG 2, which is the largest LG, spanning approximately 165 cM. In fact, all of the traits and indices mapped in this study have additive QTL or epistatic QTL on this LG. This LG therefore appears to be a hotspot for regulation of vegetative, yield and stress tolerance traits (Fig. 4).
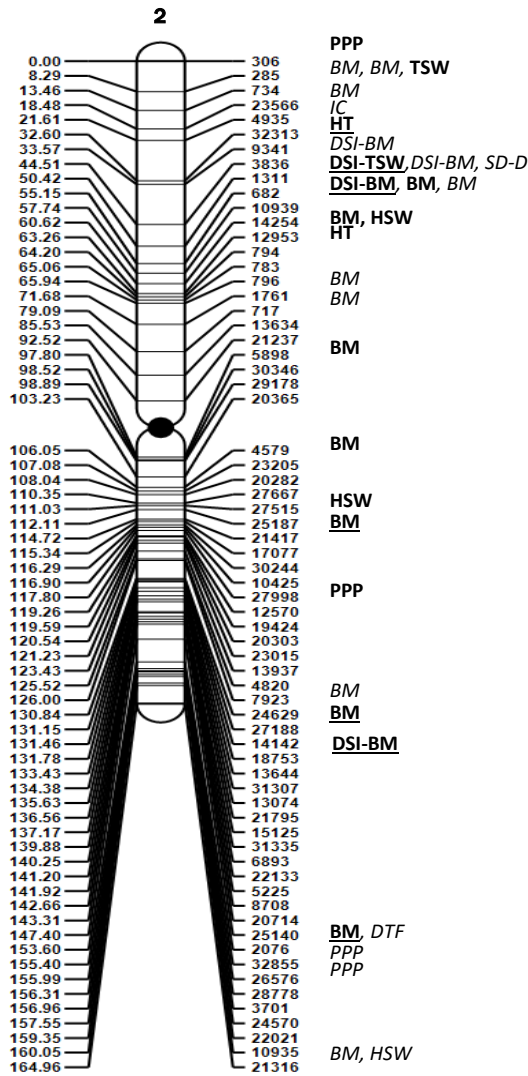
Fig. 4 LG 2 on the cowpea linkage map showing all QTL mapped. Additive QTL are in bold, with the QTL that meet the permuted threshold underlined and the QTL involved in epistatic interactions are italicized.

Quantitative trait loci have been mapped for many traits in *Vigna unguiculata* L. Walp including vegetative and yield traits (Fatokun et al. 1992; Ubi et al. 2000), disease and pest resistance (Huynh et al. 2016) (Kelly et al. 2003) (Muchero et al. 2011), heat tolerance (Lucas et al. 2013) and drought induced senescence (Muchero et al. 2009b), but this is the first mapping study to utilize restriction site associate DNA-sequencing technology and also effectively map epistatic quantitative trait loci. The use of the RAD

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sequencing identified an abundance of SNPs across the genome which would be expected to allow for better precision of QTL mapping. Epistatic mapping methods allowed for the identification of pairs of quantitative trait loci that underlay a trait to be identified, that may have gone unrecognized in other mapping methods. Identifying these quantitative trait loci and associated traits helps in the discerning the role of the loci and the discovery of genes underlying the traits.

The genetic map of this population has yet to be integrated into the other cowpea linkage maps (Menéndez et al. 1997) (Ouédraogo et al. 2002), and therefore QTL positions cannot be compared. Muchero et al., (2009) did find that maturity quantitative trait loci were not located at the same positions as drought stress QTL, which further supports the findings in this study. Integration of this high density map with the soybean and cowpea map based on expressed sequence tag (EST)-derived single nucleotide polymorphisms (Muchero et al. 2009a) would help to further increase our understanding of this crop.

3. MAPPING QTL ASSOCIATED WITH INTERVEINAL CHLOROSIS
RESISTANCE IN COWPEA

3.1 Introduction

Cowpea (*Vigna unguiculata* L. Walp) is a leguminous crop that is grown in areas around the world for its edible grain and vegetative matter. Cowpea, also known as black-eyed pea, southernpea, crowder pea, or purple hull pea, is important in the diet of many in developing countries, especially those with vegetarian diets. The grain of the cowpea is nutrient dense, containing approximately 25% protein and 4% fat with high concentrations of sodium (Na), potassium (K) and phosphorus (P), compared to other legumes (Iqbal et al. 2006). The leaves of the cowpea plant contain approximately 34% protein, and are a good supply of minerals such as K, P and Ca and phytochemicals such as carotenoids (Imungi and Potter 1983) which have been shown to play important roles in human health (Rao and Rao 2007). Like most leguminous grains, the cowpea grain also contains dietary fiber which is beneficial to human health (Messina 1999). Cowpea is also popular as livestock feed.

Due to cowpea's ability to fix nitrogen, tolerate shade, and produce a crop in often under 65 days, it makes a good crop to use in rotation or intercropping systems (Horst and Härdter 1994) (SARE 2012a) (Singh 2014). Cowpea is also tolerant of many abiotic stresses such as heat, drought, low soil P and salt, and biotic stresses such as striga, alectra, and aphids. These attributes make cowpea a great option in low input systems. Though genotypes have been identified that confer tolerance to these stresses, little work has been done in identifying genotypes with tolerance to high pH soils.

High pH soils, or alkaline soils, comprise approximately 30% of arable lands. These soils pose a problem for farmers if measures to remediate the soil or plant tolerant genotypes are not taken, because then crops will exhibit what is termed interveinal chlorosis (IC). Chlorosis refers to the yellowing of leaves of plants when micronutrients such as iron (Fe), copper (Cu), zinc (Zn), boron (B) and manganese (Mn) cannot be taken up by the plant due to insolubility in the soil. Iron is the element out of these that is used in most abundance, and when the plant is in deficit, there are detrimental effects on plant growth and

metabolism and therefore yield. The optimal pH for cowpea is 5.5 to 6.5 (SARE 2012b) and IC will begin to develop when cowpea is grown on soils with a pH at 7.5 or above (Goenaga et al. 2010).

Due to the abundance of alkaline soils and the importance of cowpea to farmers around the world, it is important to identify genotypes with IC resistance. Once genotypes are identified, then they can begin to be implemented into cowpea breeding programs. Through the use of marker assisted selection (MAS), genotypes can be screened at a more rapid pace and therefore breeding can be expedited. One of the first steps in MAS is QTL mapping. In QTL mapping, variability in a trait of interest is statistically associated to an area or areas on the genome. The markers linked to the location or locus are therefore associated with the gene or genes underlying the trait. Using MAS, genotypes can then be chosen based on the presence or absence of the marker.

Soybean is a crop of great agronomic and economic importance that also develops IC when grown on alkaline soils. Due to the synteny of cowpea with soybean (Lucas et al. 2011), markers that are identified within this crop possibly could be applied to soybean. Therefore the primary objective of this study was to map the QTL associated with alkaline soil tolerance with the long term goal of using the knowledge gained and markers identified in breeding for genotypes, both in cowpea and potentially in soybean, with superior resistance to IC.

3.2 Materials and Methods

    3.2.1 Population

Two genotypes of cowpea, 'IT98K-476-8,' an International Institute of Tropical Agriculture (IITA) breeding line and 'Golden Eye Cream' (GEC), a line released by the Texas Agricultural Experiment Station (Miller and Scheuring 2006), were identified as having differential tolerance to alkaline soils. When grown in alkaline soils, GEC becomes chlorotic, with the leaves sometimes appearing completely bleached. IT98K-476-8 on the other hand will show moderate symptoms of IC, but is able to continuously produce leaves and become less chlorotic as the plant ages, ultimately turning the shade of green that is typical for the genotype. These lines were crossed to produce the F_1 generation. Plants in the

F_1 generation were advanced to the F_8 generation by single seed descent. This F_8 population contained 184 recombinant inbred lines (RILs) which segregated for tolerance to IC.

3.2.2 Phenotyping

Each RIL and the parental genotypes were planted in 3 meter rows with 1 meter alleys in four replications in Corpus Christi, and Weslaco, TX during the summer of 2015. The soil at Corpus Christi is a Victoria clay, which is a heavy calcareous clay (Franki 1965), and the soil at Weslaco is a Willacy fine sandy loam and has a neutral to slightly alkaline pH (Turner 1982). The RIL population and parental lines were grown in RCBD with two of the reps under irrigation as control plots (C) and the other two not receiving irrigation after stand establishment to initiate drought stress (D). The plants were scored on a visual basis from one to five, with one being extremely chlorotic (white) and five being a healthy dark green.

3.2.3 Genotyping

Five to ten grams of fresh cowpea cotyledon tissue was collected from each RIL and ground with liquid nitrogen. The nucleic DNA was then isolated via a modified Doyle and Doyle method. The normalized DNA was then digested with the enzymes BamHI and MluCI. The DNA was purified to eliminate the BamHI enzyme and then Illumina adaptors ligated, then once more purified. The samples were then multiplexed and then amplified. The PCR protocol is as follows: 98°C for 30 seconds (s), then 15 cycles of 98°C for 10 s, 65°C for 30 s and 72°C for 30 s, followed by 72°C for 5 minutes. The PCR reaction was then purified. The RILS were sequenced via restriction associated DNA sequencing (RAD-seq) by BGI Americas in Cambridge MA on an Illumina Hi-Seq 2500. The analysis of sequences identified single nucleotide polymorphisms (SNPs) among the RILS and the reduced sequencing allowed for the construction of a linkage map.

3.2.4 Molecular mapping

The linkage map was created using QTL IciMapping v4.0 (Meng et al. 2015). The Kosambi function was used to convert recombination frequency to mapping distance. The markers were first binned to identify redundant markers which were correlated in the RIL population. The bins were then ordered to create the linkage map and rippled to further define the order of the map.

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The data was mapped using the QTL by environment interactions for multi-environment trials (MET) program in QTL IciMapping v4.1. The function ICIM-ADD was used to identify single effect QTL and ICIM-EPI was used to locate QTL involved in epistatic interactions. A 1,000 permutation test with P=0.05 was used to calculate the logarithm of odds (LOD) threshold. The PIN value for main effect mapping was set to 0.001 and the p value for entering a variable for the epistatic mapping was set to 0.0001.

3.2.5 Statistical analysis

ANOVA of the parental lines and RIL population was done using PROC GLM in SAS v9.4, (SAS Institute., Cary, NC, USA). Variance components and broad sense heritability (H^2) estimates were calculated on an entry mean basis using AOV in IciMapping v4.1 which utilizes the formula: $H^2 = \sigma^2 g / \sigma^2 g + \sigma^2 ge/e + \sigma^2 error/re$. In this formula, the term $\sigma^2 g$ represents the genotypic variance, $\sigma^2 ge$ represents the genotype by environmental variance, $\sigma^2 error$ represents the error variance, e was the number of environments and r was the number of reps per environment. LSmeans contrasts were performed for putative QTL using JMP Pro 12.0.1 (SAS Institute., Cary, NC, USA).

3.3 Results

3.3.1 Statistical analysis

Analysis of variance showed that the irrigation treatment had a significant interaction with the parental lines and RIL population (Table 19). Location was also a significant effect for the parents. Further analysis showed that there was no effect of treatment on IC for GEC (Table 20). Heritability estimates showed that IC is more heritable under well-watered conditions compared to moderately heritable under drought stress due to the variance arising from the environment (Table21). The RIL population demonstrated a normal distribution for this trait (Fig 5). This trait displayed transgressive segregation with scores lying above and below the mean of the parents in both locations and treatments.

Table 19 ANOVA of parental genotypes and RIL population for interveinal chlorosis ratings

	Source	DF	MS
Parents	Location	1	13.13**
	Treatment	1	13.13**
	Genotype	1	70.51**
	Location*Treatment	1	0.20
	Location*Genotype	1	0.07
	Treatment*Genotype	1	13.13**
	Location*Treatment*Genotype	1	<0.01
	Error	127	0.34
RILs	Location	1	59.53
	Treatment	1	205.51**
	rep(Location)	2	28.56**
	Genotype	169	1.80**
	Location*Treatment	1	94.30**
	Location*Genotype	169	0.63**
	Treatment*Genotype	165	0.98**
	Location*Treatment*Genotype	163	0.50
	Error	1278	0.43

* Significant at the $P=0.05$ level, ** Significant at the $P=0.01$ level

Table 20 Means and standard errors of parental genotypes and RILs at Corpus Christi (CC) and Weslaco (W) under control (C) and drought (D) conditions for interveinal chlorosis ratings

Location	CC						W					
Treatment	C			D			C			D		
IT98K-476-8	4.25	±	0.14	2.88	±	0.15**	4.75	±	0.11	3.56	±	0.18**
GEC	2.06	±	0.21	2.00	±	0.00	2.62	±	0.13	2.75	±	0.11
RILs	3.86	±	0.06	2.43	±	0.05	3.74	±	0.05	3.48	±	0.05

* Significant at the $P=0.05$ level, ** Significant at the $P=0.01$ level

Table 21 Heritability of interveinal chlorosis resistance under control and drought treatments

Control				Drought			
σ_g^2	σ_e^2	σ_{ge}^2	H^2	σ_g^2	σ_e^2	σ_{ge}^2	H^2
0.41**	0.01**	0.21**	0.69	0.12**	0.54**	0.00ns	0.46

* Significant at the $P=0.05$ level, ** Significant at the $P=0.01$ level
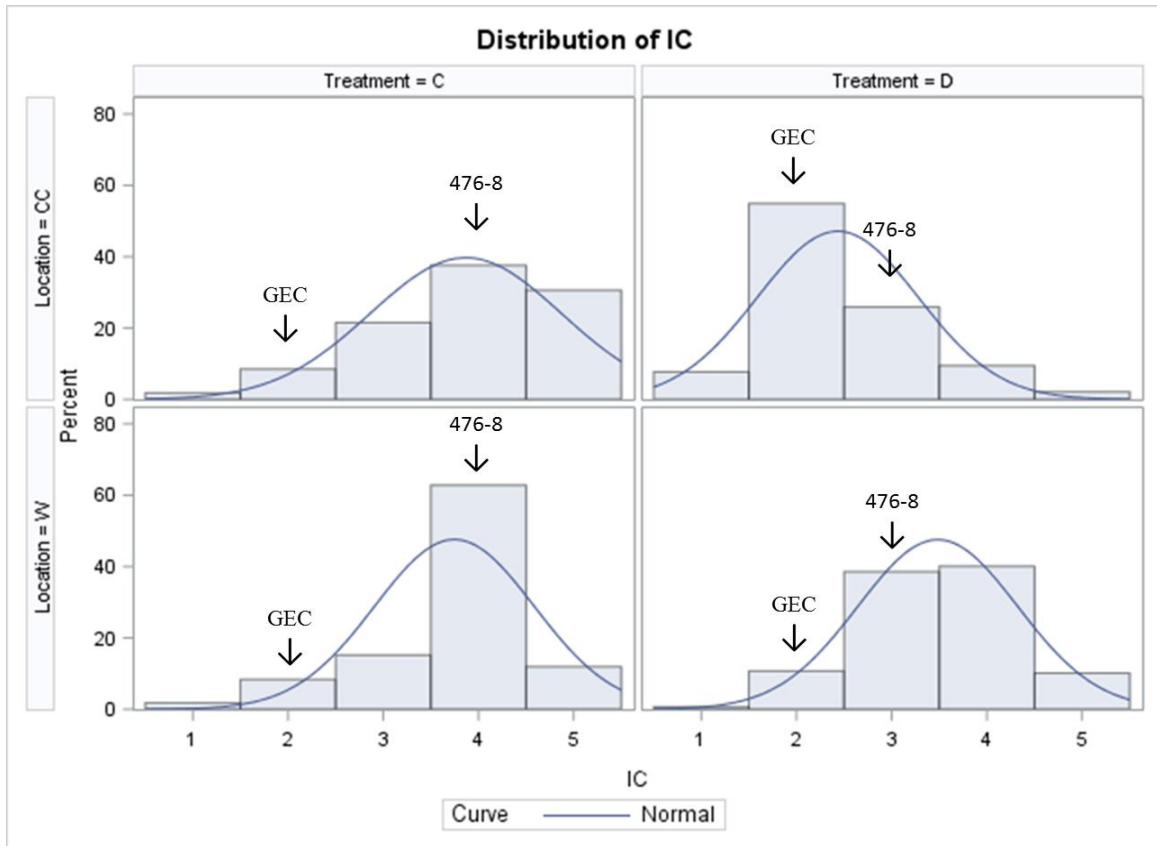
Fig. 5 Distribution of interveinal chlorosis ratings in the RIL population with means of the parental genotypes IT98K-476-8 (476-8) and GEC

3.3.2 Molecular mapping

Of the 180 genotypes, 175 produced allele calls of high quality. There was an average of 1,449,996 100-bp clean reads, with a mean genome coverage of 11.7x. Six-thousand and one SNPs were identified from the analysis of the RAD sequencing data, and from these, 4,154 SNPs were used in the construction of the linkage map. The constructed linkage map spanned 1,084.65 cM and covered 11 linkage groups, the haploid number of chromosomes for cowpea. The map had approximately one SNP marker every 0.26 cM or 149 kB. The markers were binned into 531 bins that did not co-segregate genetically.

Due to the interaction of drought with micronutrient uptake, only the control plants were mapped. The quantitative trait loci mapping identified one additive QTL that explained over 9% of the variance

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(Table 22). LSmeans contrast analysis confirmed that the left marker, 31335, was significantly correlated with an increased rating in Corpus Christi (Table 23). The right marker, 6893, was highly significantly correlated with height in Corpus Christi (refer to Chapter 2). Epistatic mapping identified four pairs of QTL which together accounted for another 15.81% of the variance (Table 24).

Table 22 Significant additive QTL for interveinal chlorosis

Trait	LOD threshold	LG	Position	Left marker	Right marker	Left CI[a]	Right CI	LOD	PVE[b]	Add[c]
IC	4.72	2	140	31335	6893	139.5	140.5	5.68	9.87	-0.15

[a] Left and right confidence intervals calculated by one LOD drop
[b] Percentage of variation explained by the QTL
[c] Additive effect of substituting the allele of IT98K-476-8 for an allele of GEC

Table 23 LSmeans contrast for markers linked to the additive QTL identified for interveinal chlorosis at LG 2 position 140

Marker		31335				6893			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
IC	CC	3.10	0.21	3.81	0.18*	3.63	0.19	3.46	0.14
IC	W	3.73	0.20	3.82	0.18	3.53	0.18	3.75	0.14
HT	CC	37.09	9.65	61.18	8.52	73.53	8.83	36.84	6.45**
HT	W	74.66	14.04	78.98	12.76	86.45	13.55	66.52	9.68
BM	CC	5.32	0.59	5.46	0.52	6.47	0.54	5.09	0.39
BM	W	6.67	0.49	6.43	0.44	7.21	0.45	6.47	0.33
TSW	CC	8.15	1.51	9.54	1.39	8.81	1.40	9.40	1.04
TSW	W	18.42	3.14	15.55	2.82	17.23	2.85	16.90	2.15
PPP	CC	5.45	1.08	6.87	0.88	7.82	0.93	6.75	0.68
PPP	W	11.68	1.85	11.99	1.68	12.08	1.69	12.66	1.24
TPW	CC	11.88	2.00	12.93	1.85	11.98	1.87	13.25	1.39
TPW	W	26.07	4.36	21.44	3.92	23.18	3.97	23.65	2.98
WTPP	CC	1.64	0.14	1.81	0.13	1.83	0.13	1.73	0.09
WTPP	W	1.84	0.12	1.91	0.10	1.90	0.11	1.78	0.08
HSW	CC	19.73	1.25	21.07	1.19	21.47	1.16	20.08	0.88
HSW	W	21.91	0.88	22.71	0.81	23.71	0.80	22.56	0.61

* Significant at the $P=0.05$ level, ** Significant at the $P=0.01$ level
[a] Allele donated by IT98K-476-8
[b] Allele donated by Golden Eye Cream

Table 24 Significant epistatic QTL for interveinal chlorosis

Trait	LOD Threshold	LG 1	Position 1	LG 2	Position 2	LOD	PVE[a]	PVE (AA)[b]	LOD (AA)[c]	Add by Add[d]
IC	7.33	2	25	6	0	7.71	2.80	1.28	2.81	-0.11
		5	30	7	35	7.46	3.69	2.15	3.85	0.14
		1	0	9	0	8.02	4.33	3.80	6.84	0.18
		2	105	9	40	9.82	4.99	3.89	7.17	0.19

[a] Percentage of variation explained by the main effect QTL

[b] Percentage of variation explained by the epistatic interaction

[c] The effect of the additive by additive interaction between two loci; negative values indicate that the interaction was between alleles originating from different parents

Table 25 LSmeans contrast for markers linked to the epistatic QTL identified for interveinal chlorosis at LG 2 position 105

Marker		20365				4579			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
IC	CC	3.53	0.16	3.61	0.16	3.64	0.24	3.75	0.24
IC	W	3.80	0.16	3.65	0.16	3.69	0.24	3.90	0.23
HT	CC	44.51	7.16	54.72	7.19	79.18	10.91	48.27	10.53**
HT	W	68.02	11.34	66.42	12.24	84.91	16.53	68.47	15.81
BM	CC	5.52	0.45	5.28	0.45	6.57	0.69	5.74	0.66
BM	W	6.52	0.37	6.59	0.38	7.52	0.57	6.66	0.55
TSW	CC	9.56	1.23	10.67	1.20	7.99	1.70	9.00	1.73
TSW	W	14.81	2.66	17.68	2.48	18.83	3.62	17.39	3.60
PPP	CC	6.88	0.79	5.92	0.80	6.04	1.19	7.17	1.14
PPP	W	12.23	1.45	12.12	1.47	13.11	2.15	13.26	2.13
TPW	CC	13.02	1.63	14.60	1.59	10.93	2.25	12.19	2.29
TPW	W	20.16	3.69	25.16	3.44	26.02	5.03	23.71	4.99
WTPP	CC	1.64	0.11	1.88	0.11	1.79	0.15	1.62	0.16
WTPP	W	1.75	0.10	1.91	0.09	1.73	0.14	1.59	0.14
HSW	CC	19.81	1.00	21.79	0.98	23.78	1.39	20.91	1.41
HSW	W	22.38	0.75	23.13	0.71	24.24	1.02	22.76	1.02

* Significant at the P=0.05 level, ** Significant at the P=0.01 level

[a] Allele donated by IT98K-476-8

[b] Allele donated by Golden Eye Cream

Table 26 LSmeans contrast for markers linked to the epistatic QTL identified for interveinal chlorosis at LG 9 position 40

Marker		13767				26343			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
IC	CC	3.69	0.20	3.71	0.17	3.61	0.22	3.77	0.24
IC	W	3.82	0.19	3.83	0.16	3.92	0.21	3.76	0.24
HT	CC	66.76	9.00	56.64	7.62	50.58	9.65	71.23	10.87
HT	W	74.63	12.68	75.09	10.70	78.76	14.38	66.59	16.93
BM	CC	6.18	0.57	5.64	0.48	5.80	0.61	6.09	0.68
BM	W	7.03	0.47	6.65	0.40	7.00	0.50	6.67	0.57
TSW	CC	9.18	1.40	8.34	1.17	9.18	1.51	9.53	1.72
TSW	W	13.43	2.94	16.60	2.47	18.36	3.34	13.81	3.63
PPP	CC	6.96	0.95	6.86	0.81	6.31	1.04	6.54	1.16
PPP	W	15.05	1.73	12.65	1.47	12.69	1.90	13.84	2.12
TPW	CC	13.05	1.86	11.30	1.55	12.04	2.00	13.31	2.28
TPW	W	18.47	4.08	22.93	3.43	25.66	4.63	19.23	5.03
WTPP	CC	1.83	0.13	1.76	0.11	1.73	0.14	1.78	0.16
WTPP	W	1.73	0.11	1.67	0.09	1.63	0.13	1.76	0.14
HSW	CC	21.96	1.14	22.27	0.96	22.75	1.24	22.12	1.42
HSW	W	23.75	0.83	23.06	0.70	23.13	0.94	23.98	1.02

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level
[a] Allele donated by IT98K-476-8
[b] Allele donated by Golden Eye Cream

Markers for IC at LG 2 position 105 also were significant for height at Corpus Christi (Table 25), but markers at LG 9 position 40 did not have a significant effect on any other trait in any location (Table 26).

3.4 Discussion

Interveinal chlorosis had a significant interaction with drought which either increased the intensity of the micronutrient deficiency therefore increasing the chlorosis, or the yellowing of the leaves due to water stress in tandem with the deficiency caused a negative shift in the rating. The skewed normal distribution in the population under irrigation in favor of the resistant parent indicates that the trait is additive but that there is an epistatic effect or effects. This was evidenced by the one additive QTL and the

four pairs of epistatic QTL that were also mapped. The additive allele was donated by the interveinal chlorosis susceptible parent, GEC. The result of having an allele donated by a susceptible parent can be explained by a possible epistatic interaction of that locus, which does not allow for expression in that parent. Lin et al. (1990), also noted the same result in mapping studies in soybean. There was no epistatic effect detected with that locus in this study or other mapping studies conducted in this population though. The QTL identified at LG 1 position 0, and LG2 position 105, which are both members of pairs of epistatic QTL, co-localize with biomass QTL found in a previous study, indicating that this QTL has a pleiotropic effect. This study confirms that interveinal chlorosis resistance is regulated by multiple genes.

When phenotyping a small population for interveinal or micronutrient deficiency, tissue samples may be taken in order to quantify the amount of micronutrients or chlorophyll in each plant. This may be effective for a small scale study, but in studies with larger populations, more cost and time efficient methods such as a rating scale or portable device methods are preferred and have proven reliable (Rodriguez de Cianzio et al. 1979). The rating scale used in this study was useful in identifying a handful of QTL underlying the trait of interest, but there is still a large percentage of the variance in data that has not been accounted for. Phenotyping the population in more environments, would increase the likelihood of identifying more significant QTL. Phenotyping for interveinal chlorosis resistance must also be done in a well-controlled environment in order to minimize the effects of environment. It should be ensured that stressors that can effect leaf color such as water stress, insects, and nitrogen deficiency are not present so that the data is an accurate reflection of the trait of interest.

4. CORRELATION OF STEM DIAMETER AND UNIFOLIATE RETENTION TO DROUGHT SUSCEPTIBILITY INDICES

4.1 Introduction

Drought tolerance is a complex trait that because of its complexity can be time and money consuming to phenotype. Using indirect measurements can be an efficient way to reduce the time and expense of phenotyping the trait of interest, but in order to be effective, what is being measured must be highly correlated with the trait of interest. Since drought tolerance is complex, researchers and breeders are constantly on the lookout for simple phenotypic traits that can help in distinguishing tolerant from susceptible genotypes. Stem diameter (Ohashi et al. 2009) (Verbree et al. 2015) and the unifoliate retention (Mai-Kodomi et al. 1999) are two traits that have been proposed to be linked to drought tolerance. The mapping population, which consists of RILs derived from the cross of IT98K-476-8 and GEC segregates for these traits. It is the objective of this study to determine if correlations exist between the unifoliate retention trait, stem diameter and drought tolerance and to map the QTL associated with these traits. The long term goal of this study is that the knowledge obtained will aid in breeding genotypes of cowpea with superior drought tolerance.

4.2 Methods and Materials

   4.2.1 Population

Two genotypes of cowpea, 'IT98K-476-8,'an International Institute of Tropical Agriculture (IITA) breeding line and, 'Golden Eye Cream,' (GEC), a line released by the Texas Agricultural Experiment Station (Miller and Scheuring 2006), were identified as having differential tolerance to drought. GEC exhibits an escape mechanism, which is exhibited by achieving its total vegetative growth and grain yield before the water stress becomes a limiting factor. In water stressed conditions, at maturity, GEC has the ability to retain healthy unifoliate leaves after all of the trifoliates have senesced. IT98K-476-8 exhibits a drought avoidance strategy by slowing above ground vegetative growth and delaying pod set

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until the water stress in relieved. This genotype also is characteristic of the Type 2 drought tolerance as characterized by Mai-Kodomo et al. (1999) whereby the unifoliates will abscise under water stress. Under water stress, IT98K-476-8 will have a reduced stem diameter, but upon rewatering, will resume growth from the main growing point, whereas GEC will retain its stem diameter, but once senesced, the genotype no longer has a viable growing point. GEC can, however, form stems, leaves and flowers at the lower plant nodes (Fig. 6).

Fig. 6 Comparison of Type 2 (Left) tolerance plants to a unifoliate retaining genotype (Right) under control and drought treatments. The Type 2 plants will lose unifoliates first and will maintain viability at the main shoot apex, while plants with healthy unifoliate retention will lose viability of the main growing point and begin to proliferate at lower nodes.

GEC and IT98K-476-8 were crossed to produce the F_1 generation. The F_1 was then advanced by single seed descent to the F_8 generation. This F_8 population contained 184 recombinant inbred lines (RILs). The population was planted in four replications under RCBD in the Southern Crops Research greenhouse (GH) on the Texas A&M campus in the fall of 2015 under a day length of 14 hours. Three seeds of each genotype were sown in one gallon plastic pots filled with Metro-mix 300 and supplemented

with *Rhizobium* inoculum and then thinned to one plant after germination. After initial stand establishment, two of the replications were watered with one cup of water two to four times per week depending on soil conditions and were considered the control treatment (C). The other two reps, for drought stress treatment (D), received exactly half that amount of water.

4.2.2 Phenotyping

4.2.2.1 Greenhouse

Days to flowering (DTF) was recorded for each plant. Ninety days after planting, the number of healthy unifoliates (UNI) on each plant were counted and scored from one to three with one being no unifoliates remaining and three being two unifoliates remaining. The stem width was measured at one cM above the potting media line with digital calipers and is hereby referred to as stem diameter (SD). Pods were counted to determine the number of pods per plant (PPP) and weighed to determine the total pod weight (TPW). Pods were hand threshed and the seeds counted and weighed to determine seeds per plant (SDPP) and total seed weight (TSW). The weight of one-hundred seeds (HSW) was determined. The vegetative matter was oven dried and the dry weight of each plant was converted into a one to 10 scale of biomass (BM). Weight per pod (WTPP) was determined by dividing the TSW by PPP.

4.2.2.2 Field Locations

The 184 lines of the RIL population and the parental lines IT98K-476-8 and GEC were planted in College Station, TX (CS) in July of 2014 and in Corpus Christi, TX (CC) in June of 2015. The lines were planted in single row, three meter plots with one meter alleys. The field was divided into four reps, with two of the reps receiving supplemental irrigation as a control (C) and the remaining two not receiving irrigation after stand establishment to initiate drought stress (D). The plants were given a foliar application of Krystal Clear® Crop Mix to alleviate micronutrient deficiencies. Plots were phenotyped in the field for height (HT) by measuring the length of the main stem from the ground to the shoot tip with a meter stick, for BM by visual scoring from one to ten with one having the least amount and 10 having the most, and for DTF. Pods were harvested separately from three plants per plot 90 days after planting. Pods from Corpus Christi were weighed to determine TPW. Pods from both locations were counted to determine PPP. The pods were then threshed to obtain the grain, which was then weighed to determine TSW per

plant. One-hundred random seeds were weighed to obtain HSW. WTPP was derived. Stem diameter and unifoliate retention were not measured in the field.

By using an equation by Fischer and Maurer (Fischer and Maurer 1978) the drought susceptibility index (DSI) was calculated for each line in the RIL population for each yield trait (Table 27). The equation $DSI= (1-Y_D/Y)(1-X_D/X)$ accounts for genotypic variability in yield potential (Y) and for varying intensities of water stress (WS) in different environments by including the second term $(1-X_D/X)$. This index has also been used in QTL mapping for heat and drought stress in other crops (Du et al. 2009) (Mason et al. 2010).

Table 27 Abbreviation, method of measurement and location of each trait phenotyped

Trait	Abbreviation	Measurement	Location
Days to flowering (days)	DTF	Number of days from planting to first bloom	CS, CC, GH,
Stem diameter	SD	Diameter of the stem	GH
Unifoliate retention	UNI	Number of unifoliate leaves present	GH
Height (cm)	HT	Length from ground to tip of main stem	CS, CC,
Biomass	BM	1-10 rank of dry weight	CS, CC, GH
Total seed weight (g)	TSW	Weight of seed from a single plant	CC, GH
Seeds per plant	SDPP	Number of seeds from a single plant	GH
Pods per plant (count)	PPP	Number of pods on a single plant	CS, CC, GH
Total pod weight (g)	TPW	Weight of all pods on a single plant	CC, GH
Weight per pod (g)	WTPP	Total pod weight divided by pods per plant	CC, GH
One-hundred seed weight (g)	HSW	Weight of one hundred random seeds	CC, CS, GH
Drought susceptibility index	DSI	Calculated for each phenotyped trait	

4.2.3 Genotyping

Five to ten grams of fresh cowpea cotyledon tissue was collected from each RIL and ground with liquid nitrogen. The nucleic DNA was then isolated via a modified Doyle and Doyle method. The normalized DNA was then digested with the enzymes BamHI and MluCI. The DNA was purified to eliminate the BamHI enzyme and then Illumina adaptors ligated, then once more purified. The samples were then multiplexed and then amplified. The PCR protocol is as follows: 98˚C for 30 seconds (s), then 15 cycles of 98˚C for 10 s, 65˚C for 30 s and 72˚C for 30 s, followed by 72˚C for 5 minutes. The PCR

reaction was then purified. The RILS were sequenced via restriction associated DNA sequencing (RAD-seq) by BGI Americas in Cambridge MA on an Illumina Hi-Seq 2500. The analysis of sequences identified single nucleotide polymorphisms (SNPs) among the RILS and the reduced sequencing allowed for the construction of a linkage map.

4.2.4 Molecular mapping

The linkage map was created using QTL IciMapping v4.0. The Kosambi function was used to convert recombination frequency to mapping distance. The markers were first binned to identify redundant markers which were correlated in the RIL population. The bins were then ordered to create the linkage map and rippled to further define the order of the map.

QTL were mapped by using the QTL by environment interactions for multi-environment trials (MET) program in QTL IciMapping v4.1. The function ICIM-ADD was used to identify single effect QTL and ICIM-EPI was used to locate QTL involved in epistatic or digenic interactions. The mean of the two replications per treatment in the greenhouse was used for mapping SD and UNI, and a 1,000 permutation test with $P=0.05$ was used to calculate the logarithm of odds (LOD) threshold. The PIN value for main effect mapping was set to 0.001 and the p value for entering a variable for the epistatic mapping was set to 0.0001.

4.2.5 Statistical analysis

ANOVA of the RIL population was done using PROC GLM in SAS v9.4, (SAS Institute., Cary, NC, USA). Pearson's correlations were carried out on all yield traits and DSIs for all traits using PROC CORR in SAS v9.4. LSmeans contrasts were performed for putative QTL using JMP Pro 12.0.1 (SAS Institute., Cary, NC, USA).

4.3 Results

4.3.1 Statistical analysis

ANOVA revealed that treatment had a highly significant effect on stem diameter and that the genotype was also highly significant. Genotype was highly significant for unifoliate retention, but treatment was not (Table 27).

Table 28 ANOVA of SD and BM under C and D treatments

	SD		UNI	
Source	DF	MS	DF	MS
Genotype	164	2.33**	163	1.04**
Treatment	1	43.61**	1	0.47
Rep	1	0.06	1	0.29
Treatment*Genotype	158	0.66**	157	0.48
Rep*Genotype	163	0.48	162	0.55
Rep*Treatment	1	0.48	1	0.19
Error	619	0.38	615	0.42

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level

Table 29 Correlation of SD and UNI to traits in the greenhouse under both treatments

	DTF	SD-C	SD-D	UNI-C	UNI-D	BM-C	PWT-C	SDPP-C	PPP-C	TSW-C	HSW-C	BM-D	PWT-D	SDPP-D	PPP-D	TSW-D	HSW-D
DTF	---	0.07	-0.06	0.04	0.09	0.32**	-0.50**	-0.58**	-0.67**	-0.50**	0.21**	0.24**	-0.27**	-0.56**	-0.60**	-0.40**	0.11
SD-C		---	0.47**	0.10	0.01	0.31**	-0.01	0.07	0.04	0.06	-0.09	0.25**	-0.03	-0.08	-0.07	0.06	0.19*
SD-D			---	0.11	0.12	0.23**	0.11	0.05	0.00	0.01	0.04	0.22**	0.17	0.06	0.06	0.16	0.20*
UNI-C				---	0.41**	0.37**	0.10	-0.07	0.01	0.03	0.14	0.12	-0.07	-0.20*	-0.09	-0.12	0.16
UNI-D					---	0.28**	-0.03	-0.20*	-0.19*	-0.80	0.26**	0.31**	-0.08	-0.21**	-0.18*	-0.10	0.23**
BM-C						---	-0.20*	-0.28**	-0.22**	-0.15	0.20*	0.40**	-0.15	-0.33	-0.23	-0.13	0.32**
PWT-C							---	0.76**	0.67**	0.80	0.01	-0.22**	0.18*	0.38**	0.26**	0.28**	-0.02
SDPP-C								---	0.77**	0.88**	-0.26**	-0.28**	0.22**	0.57**	0.38**	0.34**	-0.16
PPP-C									---	0.69**	-0.20*	-0.21**	0.20	0.46**	0.50**	0.31**	-0.09
TSW-C										---	0.05	-0.17*	0.21**	0.39**	0.32**	0.38**	0.02
HSW-C											---	0.28**	0.07	-0.27**	-0.21**	-0.09	0.32**
BM-D												---	-0.04	-0.20*	-0.13	-0.11	0.21**
PWT-D													---	0.38**	0.43**	0.32**	0.06
SDPP-D														---	0.64**	0.63**	-0.23**
PPP-D															---	0.49**	-0.08
TSW-D																---	0.44**
HSW-D																	---

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level

There was no correlation for stem diameter or unifoliate retention with days to flowering in the greenhouse. There were significant correlations between stem diameter under both treatments with biomass (Table 29). Stem diameter under both treatments was also significantly correlated to one-hundred

seed weight under drought stress. Unifoliate retention under control or well-watered treatment was significantly positively correlated to biomass under well-watered treatment and negatively correlated to seeds per plant under drought. Unifoliate retention under drought was highly significantly correlated to biomass under control treatment, negatively correlated with seeds per plant and pods per plant under C, positively correlated with biomass under drought and significantly negatively correlated with seeds per plant and pods per plant under drought. UNI-D was also positively correlated to HSW under both treatments. BM traits and yield traits were negatively correlated to each other where correlations were found with the exception of HSW. In the GH, SD-C was found to be negatively correlated with DSI-PWT, indicating that as stem diameter increased the susceptibility of the pod weight to stress increased (Table 30). SD-D was negatively correlated with DSI-SD, while SD-C was positively correlated. UNI-C was negatively correlated with DSI-HSW in CS and DSI-WTPP in CC. UNI-D was negatively correlated with DSI-BM in CS and positively correlated with DSI-TSW in CC (Table 31).

Table 30 Correlation of SD and UNI under both treatments to DSIs in the GH

	DTF	SD-C	SD-D	UNI-C	UNI-D	DSI-SD	DSI-BM	DSI-SPP	DSI-PWT	DSI-PPP	DSI-TSW	DSI-HSW
DTF	---	0.07	-0.06	0.04	0.09	0.10	0.11	-0.01	-0.02	-0.13	<0.00	-0.11*
SD-C		---	0.47**	0.10	0.01	0.62**	0.09	-0.16	-0.21*	-0.05	-0.20*	0.22*
SD-D			---	0.11	0.12	-0.35**	0.09	-0.08	-0.12	-0.09	-0.11	0.17
UNI-C				---	0.41**	-0.02	0.28**	-0.04	-0.03	-0.02	-0.03	-0.01
UNI-D					---	-0.09	0.07	-0.05	-0.03	-0.11	-0.04	<0.00
DSI-SD						---	-0.01	-0.04	-0.07	0.08	-0.07	0.13
DSI-BM								0.21*	0.19*	0.13**	0.19*	0.01
DSI-SPP								---	0.95**	0.60**	0.98**	<0.00
DSI-PWT									---	0.58**	0.94**	-0.05
DSI-PPP										---	0.59**	-0.04
DSI-TSW											---	-0.17*
DSI-HSW												---

* Significant at the P=0.05 level, ** Significant at the P=0.01 level

Table 31 Corrleation of SD and UNI measured in the greenhouse to DSI in field locations

	SD-C	SD-D	UNI-C	UNI-D	DSI-SD	DSI-HT-CS	DSI-BM-CS	DSI-PPP-CS	DSI-HSW-CS	DSI-HT-CC	DSI-BM-CC	DSI-TSW-CC	DSI-PPP-CC	DSI-TPW-CC	DSI-WTPP-CC	DSI-HSW-CC
SD-C	---	0.49**	0.10	0.02	0.59**	-0.12	-0.20*	-0.07	-0.07	-0.08	-0.09	-0.02	-0.11	0.01	0.01	0.07
SD-D		---	0.11	0.12	-0.31**	-0.17	-0.23**	-0.01	0.00	-0.19	-0.16	-0.02	-0.04	0.03	0.14	0.09
UNI-C			---	0.41**	-0.03	0.09	-0.13	-0.13	-0.26**	-0.01	0.00	0.03	-0.03	0.15	0.20*	-0.09
UNI-D				---	-0.07	0.05	-0.21*	-0.04	-0.06	0.08	0.08	0.21*	0.12	0.23	0.13	-0.02
DSI-SD					---	-0.02	-0.03	-0.07	0.00	0.11	0.02	0.01	-0.03	0.00	-0.14	0.01
DSI-HT-CS						---	0.57**	0.04	-0.27**	0.78**	0.63**	0.29**	-0.06	0.12	0.03	-0.14
DSI-BM-CS							---	-0.11	-0.01	0.45**	0.51**	-0.16	0.01	-0.19	-0.16	0.08
DSI-PPP-CS								---	-0.13	0.24*	0.04	-0.12	-0.07	-0.08	0.07	0.14
DSI-HSW-CS									---	-0.27*	-0.11	-0.02	-0.06	-0.02	0.00	-0.17
DSI-HT-CC										---	0.75**	0.47**	0.07	0.27**	-0.18	0.17
DSI-BM-CC											---	0.29**	-0.09	0.10	-0.17	-0.08
DSI-TSW-CC												---	0.79**	0.84**	0.08	-0.03
DSI-PPP-CC													---	0.54**	-0.22**	0.16
DSI-TPW-CC														---	0.32**	0.04
DSI-WTPP-CC															---	-0.20*
DSI-HSW-CC																---

*Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level

Table 32 Significant epistatic QTL for stem diameter under drought and unifoliate retention

Trait	LOD Threshold	LG 1	Position 1	LG 2	Position 2	LOD	PVE[a]	PVE (AA)[b]	LOD (AA)[c]	Add by Add[d]
SD-D	5.43	2	40	9	30	6.63	7.48	7.15	6.44	0.25
UNI	6.88	3	40	4	45	7.31	3.74	3.39	6.48	0.09
		1	20	11	45	7.52	4.01	3.75	7.01	0.09

[a] Percentage of variation explained by the main effect QTL
[b] Percentage of variation explained by the epistatic interaction
[c] The effect of the additive by additive interaction between two loci; negative values indicate that the interaction was between alleles originating from different parents

4.3.2 Molecular mapping

Of the 180 genotypes that were genotyped, 175 were able to be sequenced at a high quality. There was an average of 1,449,996 100-bp clean reads, with a mean genome coverage of 11.7x. Six thousand and one SNPs were identified from the analysis of the RAD sequencing data, and from these, 4,154 SNPs were used in the construction of the linkage map. The constructed linkage map spans 1,084.65 cM and covers 11 linkage groups, which is the haploid number of chromosomes for cowpea. The map has

approximately one SNP marker every 0.26 cM or 149 kB. The markers were binned into 531 bins that did not co-segregate genetically.

Additive mapping identified no QTLs for stem diameter or unifoliate retention. Epistatic mapping identified one QTL for the stem diameter under drought, but none for the trait under well-watered treatments or its drought susceptibility index. Epistatic mapping did identify interactions among loci though. The main effect of the epistatic QTL for SD-D mapped explains 7.48% of the phenotypic variance in the trait (Table 32). Epistatic mapping identified two pairs of QTL for UNI, which together, account for 7.75% of the variance in the trait. All of the additive by additive values for this trait are positive which indicates that the epistatic effect takes place between alleles derived from the same parental genome.

Table 33 LSmeans contrast for markers linked to the epistatic QTL identified for stem diameter under drought at LG 2 position 40

Marker		9341				3836			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
SD-C	GH	5.95	0.24	6.12	0.19	5.80	0.16	5.79	0.52
SD-D	GH	5.29	0.18	5.58	0.14	5.19	0.12	5.16	0.37
UNI	GH	1.66	0.12	1.60	0.09	1.68	0.08	1.71	0.26
HT	CC	58.69	8.19	50.22	6.20	50.36	5.41	58.58	17.00
HT	CS	41.51	4.32	40.67	3.26	40.09	2.84	45.18	8.96
HT	W	87.59	11.67	73.24	8.31	64.13	7.83	76.69	22.72
BM	CC	5.95	0.49	5.35	0.37	6.01	0.33	6.17	1.03
BM	CS	5.83	0.44	5.58	0.33	5.22	0.29	6.58	0.91
BM	W	6.96	0.41	6.78	0.31	6.85	0.27	7.66	0.86
TSW	CC	8.22	1.27	7.71	1.00	10.72	0.92	5.46	2.72
TSW	W	16.51	2.48	14.66	1.93	17.48	1.79	15.02	5.29
PPP	CC	6.73	0.84	6.62	0.70	7.40	0.53	5.95	1.91
PPP	CS	12.67	1.24	12.15	0.94	10.58	0.82	13.24	2.57
PPP	W	13.47	1.51	10.26	1.25*	13.23	1.02	8.12	3.46
TPW	CC	11.33	1.69	11.24	1.33	14.29	1.22	8.62	3.64
TPW	W	21.71	3.44	20.41	2.68	23.70	2.48	20.26	7.33
WTPP	CC	1.66	0.12	1.70	0.09	1.81	0.08	1.52	0.25
WTPP	W	1.85	0.09	1.80	0.07	1.82	0.07	1.78	0.20
HSW	CC	20.65	1.06	20.60	0.84	20.44	0.77	20.83	2.28
HSW	CS	17.65	0.78	17.88	0.58	17.91	0.51	18.45	1.58
HSW	W	23.74	0.70	22.99	0.54	22.57	0.51	23.81	1.49
DSI-HT	CC	-0.02	0.13	-0.11	0.09	-0.01	0.10	-0.12	0.24
DSI-HT	CS	0.22	0.02	0.22	0.01	0.22	0.01	0.23	0.03

Table 33 Continued

Marker		9341				3836			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
DSI-HT	W	0.01	0.07	-0.09	0.05	-0.14	0.05	-0.12	0.14
DSI-BM	CC	0.99	0.23	0.83	0.17	0.93	0.16	1.23	0.48
DSI-BM	CS	0.25	0.04	0.17	0.03	0.14	0.03	0.22	0.09
DSI-BM	W	1.11	0.29	0.83	0.22	0.80	0.20	1.30	0.59
DSI-TSW	CC	0.02	0.07	-0.03	0.06	0.13	0.05	-0.05	0.16
DSI-TSW	W	0.07	0.21	0.13	0.16	0.06	0.15	-0.05	0.46
DSI-PPP	CC	-0.05	0.06	-0.11	0.04	-0.01	0.04	-0.07	0.12
DSI-PPP	CS	-0.05	0.12	-0.02	0.09	0.04	0.07	-0.09	0.26
DSI-PPP	W	2.24	1.85	2.45	1.41	1.90	1.34	0.40	4.01
DSI-TPW	CC	0.21	0.11	0.11	0.08	0.35	0.08	0.01	0.23
DSI-TPW	W	0.06	0.30	-0.05	0.23	0.08	0.21	0.22	0.64
DSI-WTPP	CC	0.29	0.03	0.29	0.03	0.29	0.02	0.26	0.07
DSI-WTPP	W	0.41	1.38	0.88	1.05	1.22	1.00	0.16	2.96
DSI-HSW	CC	-0.0007	0.0012	-0.0001	0.0009	-0.0003	0.0009	0.0000	0.0025
DSI-HSW	CS	-0.0071	0.0035	-0.0043	0.0027	0.0029	0.0022	-0.0130	0.010*
DSI-HSW	W	0.0097	0.0038	0.0127	0.0031	0.0136	0.0026	0.0061	0.0089

* Significant at the P=0.05 level, ** Significant at the P=0.01 level
[a] Allele donated by IT98K-476-8
[b] Allele donated by Golden Eye Cream

The markers at LG 2 position 50 that were found to be significant for stem diameter under drought were also significant for PPP in Weslaco and DSI-HSW in College Station (Table 33). Both flanking markers at LG 9 position 30 were also significant for DSI-BM in CS (Table 34).

Table 34 LSmeans contrast for markers linked to the epistatic QTL identified for stem diameter under drought at LG 9 position 30

Marker		16836				4284			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
SD-C	GH	5.65	0.43	6.16	0.37	6.26	0.22	5.73	0.25
SD-D	GH	5.41	0.33	5.19	0.29	5.53	0.17	5.49	0.19
UNI	GH	1.68	0.11	1.53	0.11	1.64	0.13	1.69	0.13
HT	CC	52.33	15.24	56.59	13.30	50.52	7.97	45.35	8.39
HT	CS	36.51	8.01	39.67	6.99	41.25	4.19	36.96	4.41
HT	W	45.54	20.92	70.71	18.06	88.95	11.92	68.97	11.71
BM	CC	5.86	0.94	6.17	0.81	5.73	0.50	4.84	0.53
BM	CS	4.14	0.81	4.72	0.71	5.71	0.43	4.78	0.45
BM	W	7.49	0.76	7.02	0.66	6.74	0.40	6.40	0.42
TSW	CC	9.42	1.11	9.29	1.17	10.34	1.29	9.72	1.37
TSW	W	10.34	1.29	9.72	1.37	15.05	2.66	18.49	2.84
PPP	CC	7.38	1.53	6.76	1.34	6.22	0.80	6.70	0.85
PPP	CS	10.46	2.26	8.61	1.97	9.30	1.18	13.00	1.24
PPP	W	12.66	2.69	12.86	2.34	17.08	1.61	15.35	1.49
TPW	CC	12.95	1.47	13.03	1.54	14.40	1.71	12.96	1.81
TPW	W	25.94	3.12	19.48	3.20	18.68	3.67	27.42	3.92
WTPP	CC	1.81	0.10	1.72	0.11	1.79	0.12	1.80	0.12
WTPP	W	1.74	0.08	1.88	0.09	2.01	0.10	1.84	0.11
HSW	CC	20.39	0.91	20.69	0.96	21.55	1.06	21.20	1.13
HSW	CS	18.26	1.41	17.05	1.23	17.15	0.79	17.68	0.78
HSW	W	22.86	0.64	22.66	0.65	23.07	0.75	23.11	0.80
DSI-HT	CC	-0.07	0.10	-0.08	0.11	-0.06	0.13	-0.06	0.17
DSI-HT	CS	0.20	0.03	0.21	0.02	0.23	0.01	0.22	0.01
DSI-HT	W	-0.25	0.13	-0.09	0.11	0.06	0.07	-0.08	0.08
DSI-BM	CC	1.09	0.44	0.72	0.37	0.74	0.23	0.98	0.26
DSI-BM	CS	0.08	0.04	0.24	0.04*	0.25	0.05	0.09	0.04*
DSI-BM	W	1.13	0.53	0.60	0.46	0.80	0.28	1.07	0.31
DSI-TSW	CC	0.01	0.06	0.01	0.07	0.02	0.07	0.06	0.08
DSI-TSW	W	0.24	0.19	0.10	0.19	0.17	0.20	0.07	0.23
DSI-PPP	CC	-0.10	0.05	-0.08	0.05	-0.08	0.06	-0.09	0.06
DSI-PPP	CS	0.01	0.09	0.01	0.09	0.11	0.10	0.05	0.10
DSI-PPP	W	3.62	1.61	2.15	1.60	3.09	1.71	2.38	1.96
DSI-TPW	CC	0.19	0.09	0.21	0.10	0.24	0.11	0.25	0.11
DSI-TPW	W	-0.16	0.26	0.01	0.26	-0.10	0.29	0.03	0.32
DSI-WTPP	CC	0.30	0.03	0.30	0.03	0.33	0.03	0.30	0.04
DSI-WTPP	W	1.22	1.21	0.58	1.20	0.95	1.36	0.53	1.47
DSI-HSW	CC	-0.0004	0.0010	0.0002	0.0011	0.0004	0.0012	0.0003	0.0012
DSI-HSW	CS	0.0048	0.0030	-0.0045	0.0029	-0.005	0.0030	0.0018	0.0033
DSI-HSW	W	0.0120	0.0032	0.0165	0.0032	0.0166	0.0036	0.0109	0.0039

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level

[a] Allele donated by IT98K-476-8

[b] Allele donated by Golden Eye Cream

Table 35 LSmeans contrast for markers linked to the epistatic QTL identified for unifoliate retention at LG 1 position 20

Marker		32771				8531			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
SD-C	GH	5.64	0.26	6.41	0.28	6.44	0.24	5.63	0.29
SD-D	GH	5.18	0.18	5.81	0.20	5.79	0.18	5.15	0.20*
UNI	GH	1.93	0.12	1.41	0.12*	1.37	0.12	1.80	0.13*
HT	CC	61.68	8.15	44.44	8.14	44.85	7.92	54.88	8.20
HT	CS	48.05	4.24	35.23	4.24	35.66	4.12	42.37	4.27
HT	W	73.45	12.13	58.98	12.98	65.01	13.02	86.63	12.10
BM	CC	6.08	0.50	5.20	0.50	5.12	0.49	5.64	0.50
BM	CS	5.55	0.56	4.99	0.44	5.44	0.43	5.35	0.45
BM	W	7.08	0.42	6.56	0.42	6.27	0.41	6.63	0.42
TSW	CC	11.04	1.25	8.24	1.41	8.88	1.22	10.39	1.29
TSW	W	18.46	2.54	14.41	3.01	14.17	2.50	17.38	2.75
PPP	CC	6.32	0.85	6.83	0.88	7.73	0.81	7.15	0.92
PPP	CS	11.93	1.24	9.54	1.24	10.69	1.21	13.43	1.25
PPP	W	12.31	1.53	14.14	1.58	13.58	1.45	12.40	1.67
TPW	CC	14.29	1.69	12.17	1.89	13.26	1.69	13.68	1.71
TPW	W	27.80	3.51	17.33	4.16	17.31	3.45	26.52	3.80
WTPP	CC	1.91	0.11	1.58	0.13	1.73	0.11	1.87	0.11
WTPP	W	1.88	0.10	1.62	0.11	1.77	0.09	1.96	0.10
HSW	CC	21.96	1.03	19.22	1.16	19.39	1.00	21.44	1.06
HSW	CS	17.35	0.77	17.97	0.77	17.47	0.75	17.24	0.77
HSW	W	24.00	0.71	22.09	0.84	21.83	0.70	23.31	0.77
DSI-HT	CC	0.12	0.13	-0.21	0.12	-0.29	0.13	0.06	0.13
DSI-HT	CS	0.24	0.01	0.19	0.01*	0.20	0.01	0.24	0.01
DSI-HT	W	-0.13	0.07	-0.04	0.07	-0.02	0.07	-0.05	0.07
DSI-BM	CC	1.19	0.23	0.42	0.24	0.42	0.23	1.13	0.24
DSI-BM	CS	0.14	0.05	0.14	0.04	0.18	0.04	0.16	0.04
DSI-BM	W	1.04	0.29	0.54	0.30	0.49	0.28	1.03	0.30
DSI-TSW	CC	0.08	0.08	0.05	0.08	-0.06	0.07	0.01	0.07
DSI-TSW	W	0.77	0.19	0.07	0.21*	-0.30	0.19	0.36	0.20
DSI-PPP	CC	-0.06	0.06	-0.05	0.06	-0.15	0.05	-0.08	0.06
DSI-PPP	CS	-0.13	0.12	0.20	0.11	0.18	0.11	-0.11	0.10
DSI-PPP	W	7.89	1.65	1.92	1.81*	-1.04	1.64	4.67	1.71
DSI-TPW	CC	0.37	0.11	0.22	0.12	0.05	0.10	0.21	0.11
DSI-TPW	W	-0.84	0.27	-0.06	0.31	0.47	0.27	-0.28	0.28
DSI-WTPP	CC	0.31	0.03	0.29	0.04	0.32	0.03	0.31	0.03
DSI-WTPP	W	0.54	1.30	1.08	1.51	0.54	1.30	0.96	1.38
DSI-HSW	CC	0.0004	0.0012	-0.0010	0.0013	-0.0011	0.0011	0.0004	0.0012
DSI-HSW	CS	-0.0010	0.0037	0.0021	0.0034	0.0011	0.0033	-0.0032	0.0032
DSI-HSW	W	0.0101	0.0033	0.0191	0.0039	0.0195	0.0034	0.0071	0.0035

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level
[a] Allele donated by IT98K-476-8
[b] Allele donated by Golden Eye Cream

The markers for unifoliate retention at LG 1 position 20 were shown to have a significant effect when LSmeans contrast was performed (Table 35). This same marker was significant for DSI-HT in CS and DSI-PPP in W. The markers at LG 11 position 45 were significant HSW in CS, DSI-TSW in W and DSI-TPW in W (Table 36).

Table 36 LSmeans contrast for markers linked to the epistatic QTL identified for unifoliate retention at LG 11 position 45

Marker		27432				28532			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
SD-C	GH	6.13	0.24	6.11	0.24	6.21	0.24	6.13	0.30
SD-D	GH	5.35	0.18	5.67	0.18	5.73	0.19	5.23	0.22
UNI	GH	1.63	0.12	1.48	0.12	1.65	0.13	1.86	0.15
HT	CC	37.46	7.79	56.47	7.96	58.77	8.27	47.54	8.95
HT	CS	32.32	4.10	46.02	4.19	44.35	4.35	33.89	4.70
HT	W	61.18	11.24	80.64	11.45	85.61	12.22	62.30	12.59
BM	CC	5.08	0.48	5.63	0.49	5.53	0.51	5.36	0.55
BM	CS	4.72	0.42	5.93	0.43	5.75	0.45	4.53	0.49
BM	W	6.40	0.40	6.78	0.41	6.70	0.43	6.37	0.46
TSW	CC	9.21	1.20	8.53	1.19	10.10	1.24	11.35	1.45
TSW	W	19.19	2.57	14.11	2.55	14.94	2.66	21.60	3.08
PPP	CC	7.72	0.79	6.25	0.82	6.12	0.88	7.49	0.90
PPP	CS	13.06	1.16	10.28	1.19	9.56	1.24	11.06	1.34
PPP	W	12.83	1.47	11.66	1.57	12.15	1.53	13.09	1.89
TPW	CC	12.44	1.61	12.38	1.65	14.04	1.67	14.96	1.96
TPW	W	25.94	3.58	19.77	3.55	21.27	3.71	29.13	4.29
WTPP	CC	1.96	0.11	1.59	0.11	1.66	0.11	1.91	0.13
WTPP	W	1.87	0.10	1.74	0.10	1.84	0.10	1.92	0.12
HSW	CC	21.04	0.99	19.96	0.96	20.68	1.02	21.75	1.17
HSW	CS	19.13	0.73	16.26	0.75*	16.19	0.81	18.71	0.84
HSW	W	22.82	0.73	22.59	0.70	22.95	0.75	22.93	0.85
DSI-HT	CC	-0.16	0.13	0.04	0.14	0.02	0.13	-0.23	0.17
DSI-HT	CS	0.21	0.01	0.23	0.01	0.22	0.02	0.21	0.02
DSI-HT	W	-0.05	0.07	-0.07	0.07	-0.07	0.07	-0.06	0.08
DSI-BM	CC	0.85	0.23	0.89	0.24	0.72	0.24	0.70	0.28
DSI-BM	CS	0.11	0.04	0.19	0.04	0.20	0.05	0.08	0.05
DSI-BM	W	0.70	0.29	0.87	0.30	0.74	0.30	0.65	0.35
DSI-TSW	CC	0.01	0.07	-0.03	0.07	-0.01	0.07	0.12	0.08
DSI-TSW	W	-0.16	0.20	0.77	0.20**	0.44	0.20	-0.44	0.24*
DSI-PPP	CC	-0.15	0.05	-0.08	0.05	-0.07	0.05	-0.04	0.06
DSI-PPP	CS	-0.02	0.10	0.02	0.10	0.12	0.11	0.09	0.12
DSI-PPP	W	0.19	1.75	7.79	1.74*	5.21	1.78	-2.02	2.07*

Table 36 Continued

Marker		27432				28532			
Allele		A[a]		B[b]		A		B	
Trait	Location	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.	LS Mean	S.E.
DSI-TPW	CC	0.27	0.10	0.06	0.10	0.13	0.10	0.42	0.12
DSI-TPW	W	0.37	0.28	-0.92	0.28**	-0.47	0.28	0.78	0.33*
DSI-WTPP	CC	0.32	0.03	0.29	0.03	0.30	0.03	0.32	0.04
DSI-WTPP	W	1.01	1.35	0.54	1.34	1.06	1.37	0.47	1.60
DSI-HSW	CS	0.0015	0.0030	-0.0002	0.0030	-0.0016	0.0034	-0.0042	0.0036
DSI-HSW	W	0.0114	0.0036	0.0158	0.0036	0.0152	0.0036	0.0137	0.0046
DSI-HSW	CC	0.0010	0.0011	-0.0009	0.0011	-0.0011	0.0012	0.0011	0.0013

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level
[a] Allele donated by IT98K-476-8
[b] Allele donated by Golden Eye Cream

4.4 Discussion

Greenhouse correlations showed an association between both stem diameter and unifoliate

retention to traits expressed under control and drought treatments. There was also, in a few instances,

correlations with DSI calculated from data collected from field trials. There was no correlation between

stem diameter and unifoliate retention though.

Stem diameter had positive correlations with biomass, which is to be expected; as a plant grows,

stem size often increases in order to transport water and sugars and also support the plant. Stem diameter

in the greenhouse was found to be negatively correlated with the DSI-BM in Corpus Christi, which

indicates that as the diameter of the stem increases, the susceptibility of the biomass to drought decreases.

This increased tolerance can be explained by the plant being able to transport and store more water and

carbohydrates in its larger stem under drought, which it can then partition to the photosynthetic organs, the

leaves.

The strongest correlation was identified between the stem diameter in the greenhouse under well-

watered conditions and DSI-SD. This positive correlation indicates that as the diameter of the stem

increases, there is a larger potential for reduction of diameter under stress. The negative correlations of the

stem diameter under well-watered conditions to yield trait DSIs is indicative that this trait conferred

additional drought tolerance. There was a negative correlation of stem diameter under drought to the DSI

for stem diameter and DSI for biomass which indicated that maintenance of stem diameter under drought was associated with maintenance of biomass under drought, which was indicative of drought tolerance.

The Pearson's correlations were further supported by co-localized QTL. One of the QTL in the digenic pair of epistatic QTL for stem diameter under drought co-localized with DSI-BM on LG 2 at position 40, which was mapped in a separate drought tolerance study (Chapter 2). This confirmed that there was a correlation between these two traits and also confirmed that this locus was associated with drought tolerance mechanisms. The QTL for stem diameter under drought mapped here accounted for over 7% of the variance, and therefore a lot of variance remained unaccounted. More mapping of this trait in other locations, or with more replications, could increase the power to identify more QTL. Taking these correlations of stem diameter to drought susceptibility indices into account, it can be concluded that a large stem diameter and maintenance of the stem diameter under drought can confer tolerance to drought for biomass and some yield related traits.

Evidence from this study indicated that a larger stem diameter may be a useful trait for indirect selection for drought tolerance in a terminal drought situation, where the goal is selecting for plant survival under drought. The correlation of stem dimeter to one-hundred seed weight also indicated that this may be an indirect selection tool for yield as well. Other studies have found that stem diameter also related to pod wall weight, due to carbohydrate partitioning (Ohashi et al. 2009) (Ramirez-Vallejo and Kelly 1998) and that it was highly heritable. Therefore there is evidence that this trait would make a good trait of which to select upon. The findings in this study are consistent with other studies that examined stem diameter under water stress at the seedling stage (Verbree et al. 2015).

There were more correlations to unifoliate retention under drought than under well-watered treatment, even though ANOVA did not find the treatments significantly different. ANOVA may have not found significant difference due to population size or the moderate level water stress that was applied. This trait may also not be highly affected by environment. Analysis of the pattern of correlations with biomass to yield traits indicates that unifoliate retention acted similar to biomass. The pattern is that when unifoliates are retained and biomass is increased, then yield traits were decreased with the exception of one-hundred seed weight. Epistatic QTL for height and biomass that were mapped in a previous study co-

localized with unifoliate retention QTL at LG 3 position 40. This co-localization confirmed that there is a correlation of these traits and pleiotropy for the gene underlying the QTL. Unifoliate retention under drought is negatively associated with DSI-BM in the College Station field environment, indicating that with more unifoliates retained, the plant was more tolerant to stress induced reductions in biomass.

This study provides evidence that retention of a healthy unifoliates during water stress is an indicator of drought tolerance, though it does not find evidence that the retention itself confers tolerance. From the evidence found here, it may be hypothesized that plant is partitioning its stem tissue water and assimilated carbohydrates to the lower portion of the stem which is helping to support retention of the unifoliate, compared to the Type 2 drought stress, where water is partitioned to the growing point to maintain viability at this part of the plant and therefore unifoliates become senesced (Mai-Kodomi et al. 1999). Considering that the plants that retain their unifoliates have the ability to initiate and sustain growth at lower vegetative nodes under stress (Fig 7), it may be considered as an indicator of another type of drought tolerance.

Fig. 7 Production of flowers at lower nodes is associated with unifoliate retention

Muchero et al (2008), in terminal drought screenings of seedling drought tolerance, found that there was an association with unifoliate health to drought tolerance in one population, but not another. In the population used in this study, the unifoliate retention trait was correlated with biomass production, but if the population had been screened in a terminal drought simulation, until complete death of some genotypes, or at the seedling stage only the findings may have been different.

Growing plants until maturity in the greenhouse allowed for the evaluation of many more traits under stress than the simplified box screening method of plants at seedling stages only (Singh et al. 1999). Growing plants in the greenhouse also allowed for the control of irrigation, temperature and light, which often times cannot be controlled in the field. Though time consuming, this method allowed for the detailed phenotyping that is important when trying to establish correlation and map QTL relating to drought tolerance traits.

5. CONCLUSION

Fig. 8 QTL mapped to LG 1 through 6. Additive QTL are in bold and epistatic QTL are italicized. If the trait is repeated at the locus, it was mapped in that number of interactions.

In all, this project mapped 108 QTL at permuted LOD thresholds; with 13 being additive QTLs and the remainder being part of an epistatic interaction (Figs. 8 & 9). The mapping of epistatic traits helped to reveal the complex network of interactions that the genes associated with the QTL were involved in. The mapping of epistatic QTL also identified QTL that were involved in many interactions, indicating multiple gene regulation or pleiotropy. Mapping was also able provide more evidence of a statistical correlation between two or more traits when it was found that the QTL associated with these co-localized.\

Overall the mapping of traits for drought tolerance/susceptibility and IC resistance was successful. There are many other environments and situations that may have resulted in more QTL being mapped or more percent of the variance explained. The severity of water stress that was desired was not achieved in well-watered conditions due to abnormal amounts of precipitation. Had this not occurred, that environment would have added more power to the mapping. Though this population was a good size for a mapping population, more genotypes in a population make for better power and resolution to detect QTL and therefore would have been beneficial. In this study though, the largest hindrance to mapping drought tolerance was the parental genotypes used. It is apparent, though these two lines differ in mechanisms of tolerance, they do not differ enough in total tolerance. In addition to the lines not differing enough in overall tolerance level, they both differed in flowering time, which though not always correlated, most likely confounded the data.
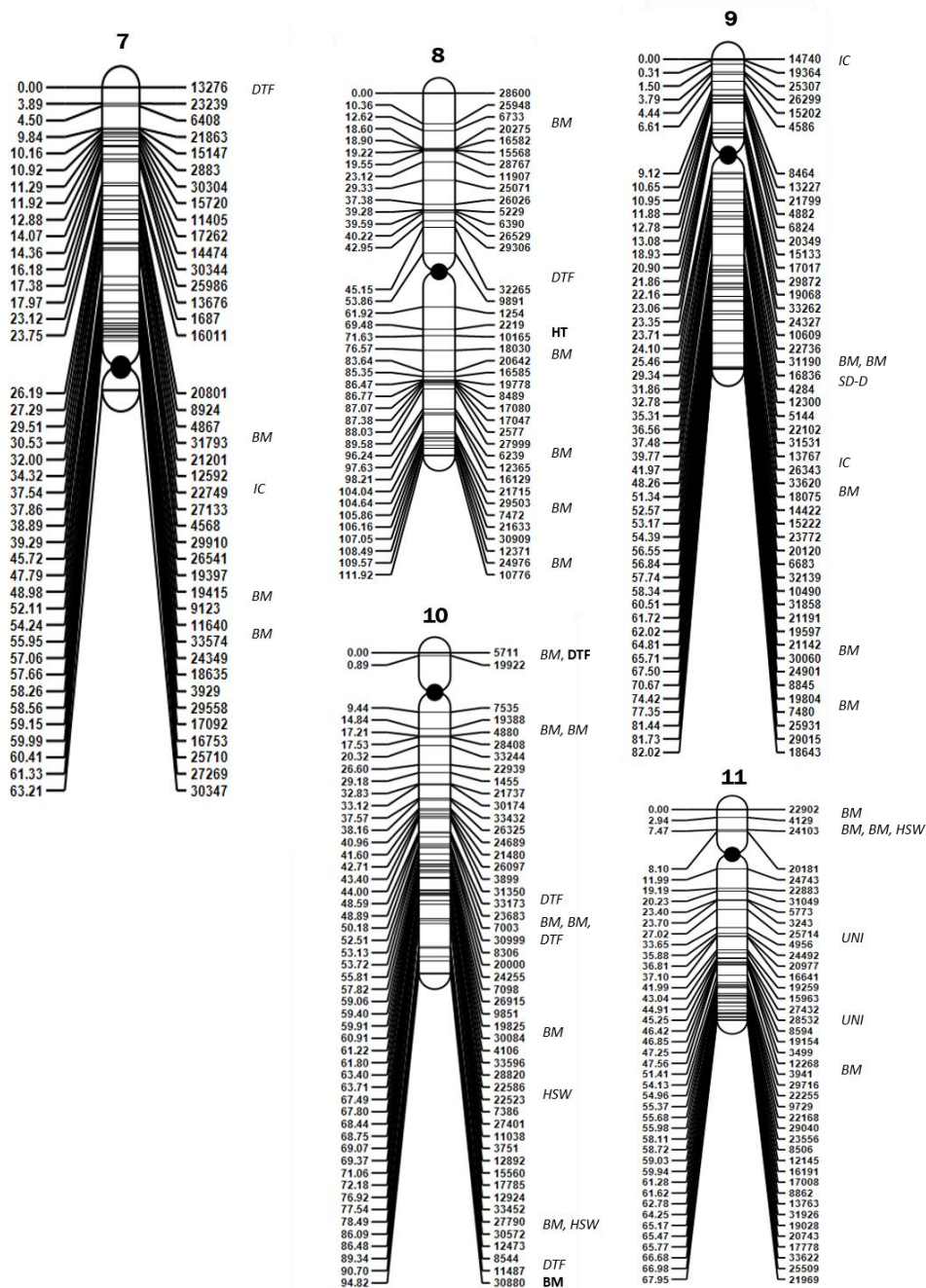
7

cM	Marker	Trait
0.00	13276	DTF
3.89	23239	
4.50	6408	
9.84	21863	
10.16	15147	
10.92	2883	
11.29	30304	
11.92	15720	
12.88	11405	
14.07	17262	
14.36	14474	
16.18	30344	
17.38	25986	
17.97	13676	
23.12	1687	
23.75	16011	
26.19	20801	
27.29	8924	
29.51	4867	
30.53	31793	BM
32.00	21201	
34.32	12592	
37.54	22749	IC
37.86	27133	
38.89	4568	
39.29	29910	
45.72	26541	
47.79	19397	
48.98	19415	BM
52.11	9123	
54.24	11640	BM
55.95	33574	
57.06	24349	
57.66	18635	
58.26	3929	
58.56	29558	
59.15	17092	
59.99	16753	
60.41	25710	
61.33	27269	
63.21	30347	

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cM	Marker	Trait
0.00	28600	
10.36	25948	
12.62	6733	
18.60	20275	BM
18.90	16582	
19.22	15568	
19.55	28767	
23.12	11907	
29.33	25071	
37.38	26026	
39.28	5229	
39.59	6390	
40.22	26529	
42.95	29306	
45.15	32265	DTF
53.86	9891	
61.92	1254	
69.48	2219	
71.63	10165	HT
76.57	18030	BM
83.64	20642	
85.35	16585	
86.47	19778	
86.77	8489	
87.07	17080	
87.38	17047	
88.03	2577	
89.58	27999	
96.24	6239	BM
97.63	12365	
98.21	16129	
104.04	21715	
104.64	29503	
105.86	7472	BM
106.16	21633	
107.05	30909	
108.49	12371	
109.57	24976	BM
111.92	10776	

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cM	Marker	Trait
0.00	14740	IC
0.31	19364	
1.50	25307	
3.79	26299	
4.44	15202	
6.61	4586	
9.12	8464	
10.65	13227	
10.95	21799	
11.88	4882	
12.78	6824	
13.08	20349	
18.93	15133	
20.90	17017	
21.86	29872	
22.16	19068	
23.06	33262	
23.35	24327	
23.71	10609	
24.10	22736	
25.46	31190	BM, BM
29.34	16836	SD-D
31.86	4284	
32.78	12300	
35.31	5144	
36.56	22102	
37.48	31531	
39.77	13767	IC
41.97	26343	
48.26	33620	BM
51.34	18075	
52.57	14422	
53.17	15222	
54.39	23772	
56.55	20120	
56.84	6683	
57.74	32139	
58.34	10490	
60.51	31858	
61.72	21191	
62.02	19597	
64.81	21142	BM
65.71	30060	
67.50	24901	
70.67	8845	
74.42	19804	BM
77.35	7480	
81.44	25931	
81.73	29015	
82.02	18643	

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cM	Marker	Trait
0.00	5711	BM, DTF
0.89	19922	
9.44	7535	
14.84	19388	
17.21	4880	BM, BM
17.53	28408	
20.32	33244	
26.60	22939	
29.18	1455	
32.83	21737	
33.12	30174	
37.57	33432	
38.16	26325	
40.96	24689	
41.60	21480	
42.71	26097	
43.40	3899	
44.00	31350	
48.59	33173	DTF
48.89	23683	BM, BM,
50.18	7003	DTF
52.51	30999	
53.13	8306	
53.72	20000	
55.81	24255	
57.82	7098	
59.06	26915	
59.40	9851	
59.91	19825	
60.91	30084	BM
61.22	4106	
61.80	33596	
63.40	28820	
63.71	22586	
67.49	22523	HSW
67.80	7386	
68.44	27401	
68.75	11038	
69.07	3751	
69.37	12892	
71.06	15560	
72.18	17785	
76.92	12924	
77.54	33452	
78.49	27790	BM, HSW
86.09	30572	
86.48	12473	
89.34	8544	
90.70	11487	DTF
94.82	30880	**BM**

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cM	Marker	Trait
0.00	22902	BM
2.94	4129	BM, BM, HSW
7.47	24103	
8.10	20181	
11.99	24743	
19.19	22883	
20.23	31049	
23.40	5773	
23.70	3243	
27.02	25714	UNI
33.65	4956	
35.88	24492	
36.81	20977	
37.10	16641	
41.99	19259	
43.04	15963	
44.91	27432	
45.25	28532	UNI
46.42	8594	
46.85	19154	
47.25	3499	
47.56	12268	BM
51.41	3941	
54.13	29716	
54.96	22255	
55.37	9729	
55.68	22168	
55.98	29040	
58.11	23556	
58.72	8506	
59.03	12145	
59.94	16191	
61.28	17008	
61.62	8862	
62.78	13763	
64.25	31926	
65.17	19028	
65.47	20743	
65.77	17778	
66.68	33622	
66.98	25509	
67.95	21969	

Fig. 9 QTL mapped to LG 7 through 10. Additive QTL are in both and epistatic QTL are italicized. If the trait is repeated at the locus, it was mapped in that number of interactions.

The QTL that were mapped are associated with SNP markers. The regions that contain the SNPs associated with the traits can be cloned and the genes further studied. The ultimate goal of mapping studies

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is that the markers associated with the traits can eventually be used in marker assisted breeding, but for this crop, that application is most likely further down the road than it would be for more simple traits in more utilized crops, unless the QTL was linked to a gene that had large positive effects while under drought stress. Therefore the next logical step in this project would be to integrate it with other cowpea maps and the integrated soybean and cowpea map. This integration would help to confirm the QTLs found among projects. Since the integrated soybean and cowpea map has known genes mapped, it could possibly help with causal gene association.

Breeding with the aid of MAS is very useful in simply inherited traits such as flower color, or disease resistance, but the complexity of drought stress makes it less effective. Nonetheless, scientists try to simplify drought tolerance, and boil it down to one or a few traits and with the goal in mind of identifying a marker that will confer drought tolerance. While there are genes that can be selected for, there are no genes associated with drought tolerance that confer enough tolerance that all other drought tolerance traits can be ignored. Stem diameter for example, has proven to be a reliable method of screening for biomass maintenance during water stress, but this trait does not show strong correlation to yield under water stress. Data from this study suggested that there is a pleiotropic tradeoff between BM and yield, therefore, if the goal was to breed for yield under non terminal drought, screening first for stem diameter may be a waste of time.

With this is mind, it is apparent that more work needs to be done regarding this study to aid the project in realizing its long term goal of being useful in breeding for genotypes that have superior interveinal chlorosis resistance and drought tolerance. Using the IT98K-476-8 x GEC RIL population, more drought related physiological traits such as gas exchange, water use efficiency, or chlorophyll fluorescence can be phenotyped and mapped in order to identify the relation to the DSIs and other traits currently mapped. This population can also be screened at the seedling stage for stem diameter and unifoliate retention to see if correlations exist among this stage and cowpea at maturity. Some of the future plans for this study may be to develop, phenotype and genotype other biparental populations in order to identify common QTL. All of these options would further increase the knowledge that would eventually aid in efforts by breeders to create superior genotypes of cowpea for a growing population.

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SUPPLEMENTAL TABLES FOR DROUGHT SUSCEPTIBILITY MAPPING

Table A-1 ANOVA of parental genotypes for all traits by location

Source	CC		CS		W	
	DF	MS	DF	MS	DF	MS
DTF						
Treatment	1	31.64	1	224.12**	1	17.02
Genotype	1	2983.89**	1	984.15**	1	907.52**
Treatment*Genotype	1	66.02	1	0.02	1	0.39
Error	63	43.15	64	13.88	63	15.28
HT						
Treatment	1	3282.34**	1	538.39**	1	5389.23**
Genotype	1	5877.78**	1	502.74**	1	137231.83**
Treatment*Genotype	1	1757.01**	1	0.74	1	4054.13*
Error	63	115.93	63	14.08	35	737.02
BM						
Treatment	1	42.25**	1	181.41**	1	234.47**
Genotype	1	189.06**	1	114.89**	1	10.16**
Treatment*Genotype	1	2.25	1	20.53**	1	1.72*
Error	63	1.22	59	3.69	63	0.47
TSW						
Treatment	1	80.79*	---	---	1	33.35
Genotype	1	25.83	---	---	1	1345.06**
Treatment*Genotype	1	19.67	---	---	1	124.32
Error	58	15.6	---	---	63	47.44
PPP						
Treatment	1	505.73**	1	131.95	1	0.07
Genotype	1	199.95**	1	53.9	1	289.71**
Treatment*Genotype	1	83.54**	1	18.5	1	54.7
Error	58	11.64	60	43.9	63	23.98
TPW						
Treatment	1	221.51**	---	---	1	28.62
Genotype	1	0.15	---	---	1	966.17**
Treatment*Genotype	1	65.96	---	---	1	283.36
Error	58	23.1	---	---	63	89.68
WTPP						
Treatment	1	0.22	---	---	1	0.16
Genotype	1	7.91**	---	---	1	0.08
Treatment*Genotype	1	5.84**	---	---	1	<0.00
Error	58	0.29	---	---	63	0.06
HSW						
Treatment	1	12.00*	1	15.99**	1	19.11**
Genotype	1	235.39**	1	635.89**	1	331.71**
Treatment*Genotype	1	51.00**	1	2.52	1	24.07**
Error	34	2.24	47	2.37	40	1.56

* Significant at the P=0.05 level, ** Significant at the P=0.01 level

Table A-2 ANOVA of RILs for all traits by location

Source	CC DF	CC MS	CS DF	CS MS	W DF	W MS	GH DF	GH MS
DTF								
Treatment	1	112.16	1	1885.54**	1	0.99	1	7.26
Genotype	164	208.99**	172	172.10**	163	215.52**	165	221.47**
Treatment*Genotype	143	51.79**	156	48.14**	155	32.59**	153	31.60
Error	233	36.25	608	8.06	605	17.72	563	33.98
HT								
Treatment	1	60762.00**	1	10978.87**	---	---	---	---
Genotype	172	1367.67**	174	492.74**	---	---	---	---
Treatment*Genotype	155	1343.36**	154	386.48**	---	---	---	---
Error	590	105.31	638	37.20	---	---	---	---
BM								
Treatment	1	1220.88**	1	544.05**	1	584.83**	---	---
Genotype	172	6.54**	171	4.80**	173	4.73**	---	---
Treatment*Genotype	156	5.79**	128	4.48**	156	4.88**	---	---
Error	598	1.31	526	1.07	649	2.99	---	---
TSW								
Treatment	1	708.47**	---	---	1	581.55	---	---
Genotype	145	48.49**	---	---	164	258.86*	---	---
Treatment*Genotype	128	24.47	---	---	140	223.78	---	---
Error	447	33.78	---	---	519	201.11	---	---
PPP								
Treatment	1	237.96**	1	896.29**	1	17569.20**	---	---
Genotype	174	29.42	170	44.09**	173	241.13	---	---
Treatment*Genotype	120	34.30	129	42.38*	139	258.25	---	---
Error	451	33.92	549	32.50	518	271.11	---	---
TPW	1	5647.94**						
Treatment	146	50.09	---	---	1	8562.14**	---	---
Genotype	127	38.66	---	---	164	301.61**	---	---
Treatment*Genotype	449	44.62	---	---	141	240.81	---	---
Error			---	---	523	206.87	---	---
WTPP								
Treatment	1	95.85**	---	---	1	160.89**	---	---
Genotype	146	0.19	---	---	163	0.12	---	---
Treatment*Genotype	125	0.31**	---	---	140	0.24**	---	---
Error	447	0.18	---	---	520	0.12	---	---
HSW								
Treatment	1	45.12**	1	52.13**	1	798.80**	---	---
Genotype	144	28.63**	169	19.60**	164	21.50**	---	---
Treatment*Genotype	125	13.97**	120	21.08**	139	6.38**	---	---
Error	395	3.88	513	3.67	513	3.67	---	---

* Significant at the P=0.05 level, ** Significant at the P=0.01 level

Table A-3 LSD of of parental genotypes for all traits

Genotype	DTF t Grouping	DTF Mean	DTF N	HT Grouping	HT Mean	HT N	BM Grouping	BM Mean	BM N	TSW Grouping	TSW Mean	TSW N	PPP Grouping	PPP Mean	PPP N	TPW Grouping	TPW Mean	TPW N	WTPP Grouping	WTPP Mean	WTPP N	HSW Grouping	HSW Mean	HSW N
476	A	49.34	96	A	60.4	80	A	5.79	96	B	8.89	60	B	8.5	91	B	13.42	60	A	1.85	60	B	16.26	24
GEC	B	39.68	97	B	25.76	84	B	4.12	92	A	14	63	A	10.4	93	A	16.96	63	B	1.5	63	A	23.54	24

Table A-4 LSD of treatments for all traits among parental genotypes

Treatment	DTF t Grouping	DTF Mean	DTF N	HT Grouping	HT Mean	HT N	BM Grouping	BM Mean	BM N	TSW Grouping	TSW Mean	TSW N	PPP Grouping	PPP Mean	PPP N	TPW Grouping	TPW Mean	TPW N	WTPP Grouping	WTPP Mean	WTPP N	HSW Grouping	HSW Mean	HSW N
C	ns	---	---	A	49.03	82	A	5.6	94	ns	---	---	A	10.81	94	ns	---	---	ns	---	---	ns	---	---
D	ns	---	---	B	36.29	82	B	4.35	94	ns	---	---	B	8.05	90	ns	---	---	ns	---	---	ns	---	---

Table A-5 LSD of of RILS genotypes for all traits

Treatment	DTF t Grouping	DTF Mean	DTF N	HT Grouping	HT Mean	HT N	BM Grouping	BM Mean	BM N	TSW Grouping	TSW Mean	TSW N	PPP Grouping	PPP Mean	PPP N	TPW Grouping	TPW Mean	TPW N	WTPP Grouping	WTPP Mean	WTPP N	HSW Grouping	HSW Mean	HSW N
C	A	46.83	1129	A	49.51	1564	A	5.75	917	A	9.53	237	B	7.03	237	A	18.96	516	A	1.8	516	A	20.29	798
D	A	46.78	1192	B	34.8	1508	B	3.39	859	B	6.79	211	A	8.92	215	B	10	458	B	0.71	453	B	18.78	618

Table A-6 LSD of genotypes of all traits by location

	CC			CS			W		
Genotype	t Grouping	Mean	N		Mean	N		Mean	N
DTF									
476	A	53.1	32	A	45.06	32	A	49.88	32
GEC	B	39.4	32	B	37.33	32	B	42.34	32
HT									
476	A	60.4	80	A	33.01	32	A	152.4	16
GEC	B	25.8	84	B	27.40	32	B	28.13	20
BM									
476	A	6	32	A	4.75	32	A	5.48	32
GEC	B	2.56	32	B	2.07	32	B	4.69	32
TSW									
476	ns	---	---	---	---	---	A	19.88	32
GEC	ns	---	---	---	---	---	B	10.72	32
PPP									
476	B	6.17	28	ns	---	---	B	8.63	32
GEC	A	9.76	31	ns	---	---	A	12.88	32
TPW									
476	ns	---		---	---	---	B	16.43	32
GEC	ns	---		---	---	---	A	24.2	32
WTPP									
476	A	1.85	28	ns	---	---	ns	---	---
GEC	B	1.06	31	ns	---	---	ns	---	---
HSW									
476	B	16.3	24	B	16.26	24	B	18.54	20
GEC	A	23.5	24	A	23.54	24	A	24.01	21

Table A-7 LSD of treatments of all traits by location

	CC			CS			W		
Genotype	t Grouping	Mean	N		Mean	N		Mean	N
DTF									
C	ns	---	---	A	42.91	32	ns	---	---
D	ns	---	---	B	39.31	32	ns	---	---
HT									
C	A	39.4	32	A	33.11	32	A	94.48	18
D	B	25.1	32	B	27.31	32	B	72.23	18
BM									
C	A	5.09	32	A	5.09	32	A	7.00	32
D	B	3.47	32	B	1.73	32	B	3.17	32
TSW									
C	A	8.41	32	---	---	---	ns	---	---
D	B	6.19	27	---	---	---	ns	---	---
PPP									
C	A	10.7	32	ns	---	---	ns	---	---
D	B	4.9	27	ns	---	---	ns	---	---
TPW									
C	A	11.5	32	---	---	---	ns	---	---
D	B	7.67	27	---	---	---	ns	---	---
WTPP									
C	ns	---	---	---	---	---	ns	---	---
D	ns	---	---	---	---	---	ns	---	---
HSW									
C	B	20.2	16	A	20.48	24	B	20.66	17
D	A	21.4	19	B	16.26	24	A	24.01	21

Table A-8 Variance component estimates for the random effects, location and days to flower, in the Residual Maximum Likehood (REML) model for biomass ratio, one-hundred seed weight, height and pods per plant

	HT			BMR			HSW			PPP		
	Variance	S.E.	% of total	Variance	S.E.	% of total	Variance	S.E.	% of total	Variance	S.E.	% of total
Location	280.69	284.26	27.71	0.28	0.75	21.66	4.14	4.33	27.12	31.72	32.07	19.76
DTF	<0.00	0.03	0.00	<0.00	<0.00	0.01	<0.00	<0.00	0.00	<0.00	0.01	<0.00
Residual	732.26	30.63	72.29	2.70	0.10	78.32	11.11	0.46	72.88	128.83	5.29	80.24

Table A-9 Variance component estimates for the random effects, location and days to flower, in the REML model for total seed weight, total pod weight and weight per pod

	TSW			TPW			WTPP		
	Variance	S.E.	% of total	Variance	S.E.	% of total	Variance	S.E.	% of total
Location	52.23	74.29	29.95	62.59	95.25	31.52	<0.00	<0.00	0.29
DTF	<0.00	<0.00	0.00	<0.00	<0.00	0.00	<0.00	<0.00	<0.00
Residual	122.20	6.21	70.05	135.99	6.89	68.48	0.19	<0.00	99.72

Table A-10 Variance component estimates for the fixed effects, treatment, entry and rep in the REML model for all traits

	HT		BMR		HSW		PPP		TSW		TPW		WTPP	
	DF	P Value	DF	P Value	DF	P Value	DF	P Value	DF	P Value	DF	P Value	DF	P Value
Treatment	1	<.00**	1	<.00**	1	-	1	<.00**	1	0.07	1	-	1	<.00**
Entry	172	<.00**	172	<.00**	171	-	171	0.99	159	<.00**	159	-	158	0.18
Rep	1	0.54	1	<.00**	1	-	1	0.89	1	<.00**	1	-	1	0.02*

* Significant at the *P*=0.05 level, ** Significant at the *P*=0.01 level