5-2015

Genetic Mapping of Yield and Normalized Difference Vegetative Index in Soft Red Winter Wheat (Triticum aestivum L.)

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Crop, Soil & Environmental Sciences

by

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University of Arkansas
Bachelor of Science in Animal Science, 2012

May 2015
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This thesis is approved for recommendation to the Graduate Council.

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Abstract

Wheat is the most widely cultivated cereal crop, being grown on 17% of the global crop land and as such must be adapted to an array of environmental stresses. In order to become a variety, wheat breeding lines must be tested across a range of environments for both high productivity and stability. Quantitative trait loci (QTL) mapping and spectral reflectance indices are tools that can aid plant breeders in the selection of superior lines. Soft red winter wheat accounts for 20% of the total wheat production in the United States, being grown in the southeastern U.S. predominantly along the Mississippi River. However, there are currently no reports of QTL associated with grain yield and test weight in U.S. soft red winter wheat. The objective of this study was to identify QTL associated with grain yield, test weight and related traits to aid breeders in the southeastern U.S to better understand the genetic control of adaption to this region that can lead to higher production and producer return. This study was also aimed to assess the ability of normalized difference vegetative index (NDVI) to monitor changes in crop development over the growing season as well as to identify QTL influencing these changes.

A recombinant inbreed line (RIL) population derived from a cross between two elite cultivars, 'Pioneer 26R61' and 'AGS 2000', was grown in six different testing sites from 2011-2014 for a total of twelve site-years. A randomized complete block design was used with two replications per location. Mean grain yield for the RILs ranged from 339 g m^{-2} to 716 g m^{-2} and test weight from 69 kg hl^{-1} to 80 kg hl^{-1}. A total of 42 QTLs were detected for yield, test weight, heading date and height. Eleven yield QTL were identified, explaining from 1.8 to 8.5% of the phenotypic variation and contributed by both of the parental lines. Yield QTL explaining the most variance were located on chromosome 5B and were also associated with the favorable allele from AGS2000 for early heading. Eight QTL were identified for test weight, with the largest effect locus on 5D explaining 7.1% of the phenotypic variance.

For the NDVI study, NDVI measurements were repeated on multiple days throughout the growing season with at least one measurement taken during vegetative and grain-filling stages at seven Arkansas site-years. Based on the accumulated growing degree days, NDVI measurements were grouped into seven development stages. In addition, vegetative biomass samples were harvested during early plant development and biomass at maturity was estimated from 50 tillers harvested at ground level prior to whole plot harvest. Genetic variation and heritability of NDVI increased throughout the growing season as did correlations between NDVI and biomass or yield. Significant correlations ranged from r = -0.32 to 0.37 for NDVI development stages with yield, biomass at maturity and vegetative biomass. For individual environments, particularly those that had low production, correlations were found to be as high as r = 0.72 for late season measurements of NDVI and yield. QTL for NDVI were found to be highly pleiotropic and were clustered in 14 genome regions across 11 of the 21 wheat chromosomes. Six of the 14 regions co-localized for both NDVI and biomass, with individual QTL explaining up to 14.7% of the phenotypic variation for NDVI.

Results presented here can aid breeders in future development of high yielding cultivars through marker assisted breeding and in targeting growth and development to meet the demands of a diverse range of growing environments.

Acknowledgments

I would like to thank my major advisor, Dr. R. Esten Mason for guidance, encouragement and the enthusiasm he brings to work that ultimately persuaded me to pursue my degree. I would like to thank my committee members, Dr. Larry Purcell, Dr. Kristofor Brye and Dr. Edward Gbur for their patience, understanding and advice.

I would also like to thank the CSES department for the employment as an undergraduate that introduced me to the world of wheat breeding and genetics. I would like to thank all the CSES department staff for always making sure I had the things done that needed to be done. I am incredibly thankful for my fellow members of the Wheat Breeding and Genetics Lab. Memories made planting, harvesting and processing samples will last a lifetime. I wish you guys all the best!

Finally, I want to thank my friends and family for always believing in me and my desire to make a difference. You were always there for the ups and the downs. Words cannot describe how much I appreciate and love you guys for all the things you have done.

Dedication

"The two most important days in your life are the day you're born and the day you find out why." – Mark Twain

To the wonderful people in my life that remind me every day that I can make a difference in fighting world hunger. Your encouragement is what drives me to never give up and keep pushing forward. I cannot thank you enough.

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Chapter I

Introduction and Literature Review

INTRODUCTION

Wheat is cultivated on 17% of global crop land and contributes more than 20% of total calories consumed worldwide. Being the most widely cultivated cereal crop, wheat must be adapted to a wide range of environments and abiotic stresses, including heat, drought, soil waterlogging and others that collectively decrease wheat yield and end-use quality. Due to high genotype by environmental interaction for yield, breeding programs must test promising lines across a range of environments in order to identify genotypes that are both highly productive and stable in terms of yield. Both genomic and physiological tools are available to aid breeders in the selection of superior lines. The use of remote sensing for field phenotyping has become increasingly popular in recent years as it allows researchers to monitor crop growth and predict crop performance non-destructively. Spectral reflectance indices (SRIs) are measured using a spectral radiometer and detect changes in the reflectance spectrum of the crop canopy which can be translated into differences in phenotype among segregating genotypes. Normalized difference vegetative index (NDVI) is a SRI widely used in crops to monitor plant health and vigor and predict biomass and yield.

Genetic mapping can be used to identify genomic regions associated with traits of interest, such as yield, which can lead to development of markers linked to promising quantitative trait loci (QTL) that can be used for marker-assisted breeding. Currently, there are no reports of QTL associated with grain yield in U.S soft red winter wheat (SRWW), and this lack of knowledge is a constraint to breeders. The aim of this proposal is to combine measurements of NDVI with genetic mapping of yield and yield components to better understand the genetic control of wheat yield across the southeastern U.S. where SRWW is grown. The results of this study will provide information on the wheat genomic regions

associated with yield, which yield components contribute to total yield and determine the utility of NDVI as a tool for selection of biomass and yield. Collectively these results will provide breeders with tools that can aid in selecting superior lines adapted to the SRWW growing region.

OBJECTIVES

The main objective of this proposal is to identify QTL associated with grain yield in soft red winter wheat and determine the significance of these QTL across testing locations in the southeastern United States. Secondly, yield QTL will be broken down by measuring yield components and using NDVI as a proxy for biomass at Arkansas testing sites. The contribution of yield components and NDVI to total yield will be determined by analyzing the phenotypic correlations and the co-localization of QTL for these traits within the genome. The specific objectives are as follows:

Objective 1: Determine the genetic variation for yield, yield components and NDVI present in a soft red winter wheat mapping population derived from two elite cultivars. This objective was accomplished through cooperative regional testing of a recombinant inbred line (RIL) population in six locations over two seasons. Testing sites were located in Arkansas (3 sites), Georgia, Louisiana, and Texas. Total grain yield and agronomic data will be collected in all locations. Data for yield components and NDVI will be collected in the Arkansas testing sites.

Objective 2: Identify QTL associated with grain yield, yield components and NDVI and determine the significance of detected QTL across regional testing locations. Phenotypic

data collected in Objective 1 was used to detect QTL using a dense single nucleotide polymorphism (SNP) based genetic map. The SNP based genetic map enabled the identification of candidate genes underlying detected QTL. The working hypothesis for this objective is that the parental lines used in this study have different mechanisms of adaptation and these mechanisms will segregate within the RIL population.

Objective 3: Determine the genetic control of biomass accumulation using repeated measurements of NDVI over time. To accomplish this objective, weekly NDVI measurements were taken beginning in the early vegetative stage to maturity and used to investigate changes in biomass over time. The relationship between NDVI and grain yield and the associated genomic regions was also be investigated. The working hypothesis for this objective is that NDVI serves as a measurement of total canopy biomass and can therefore be used to monitor plant growth over time.

LITERATURE REVIEW

Wheat History and Importance

Wheat is a cereal crop that can be divided into two major cultivated species, *Triticum aestivum* and *Triticum durum*. *Triticum aestivum* is widely cultivated due to its flour properties for making bread and other baked goods, while *Triticum durum* is utilized for making pasta. Wheat was first cultivated 10,000 years ago in the south-eastern part of Turkey. This early cultivation consisted of einkorn (AA) and emmer (AABB) wheat. The first appearance of bread wheat (AABBDD)

was not recorded until 9,000 years ago after wheat spread to the Near East. Bread wheat has a starch content around 60-70%, and a low protein content of 8-15%. The high starch and low protein content makes bread wheat a desired food for humans and a nutritional feed supplement for animal production (Shewry 2009). Wheat accounts for 20% of total consumed calories by humans (Brenchley et al. 2012) and is also a good source of minerals for people throughout the world, contributing up to 15% of the recommended daily intake of iron and 11% of the daily intake of zinc (Shewry 2009).

Wheat Genetics and Genomic Resources

Bread wheat has a large complex genome when compared to other crops, making sequencing and ordering of the genome a difficult task for researches. *Triticum aestivum* is a hexaploid species (2n = 6x = 42, AABBDD) with a genome size of 15,961 Mb (Peng et al. 2011). Bread wheat is the result of hybridization between cultivated tetraploid emmer wheat (AABB, *Triticum dicoccoides*) and diploid goat grass (DD, *Aegilops tauschii*) (Brenchley et al. 2012). *Triticum durum* is a tetraploid species (2n = 4x = 28, AABB) with a genome of 11,660 Mb and developed from the selection of free-threshing forms of emmer wheat (Peng et al. 2011).

Given the importance of wheat as a food source around the world, there have been worldwide efforts to develop genomic resources to assist in genetic improvement. Recent work by Brenchley et al. (2012) used whole-genome shotgun sequencing to sequence the bread wheat transcriptome, including 96,000 gene assemblies. While microsatellite or simple sequence repeat markers were once the marker of choice for wheat research (Kalia et al. 2011), next generation sequencing techniques have identified an abundance of single nucleotide polymorphism (SNP)

markers distributed throughout the genome (Cavanagh et al. 2013; Poland et al. 2012). Though sequencing and ordering of a full hexaploid wheat genome sequence is still ongoing, successful efforts have been made to draft genome sequences for the individual genomes of ancestral species. The recently completed draft genomes for both *Triticum urartu* (Ling et al. 2013) and *Aegilops tauschii* (Jia et al. 2013), wild ancestors of the A and D genomes, respectively, will serve as important tools for wheat genomics and genetic improvement going forward.

Wheat production around the world

Wheat is the number one crop in terms of overall grain production around the world (Peng et al. 2011), with 95% of the total wheat area comprised of bread wheat and the remaining 5% durum wheat (Shewry 2009). The top five producers of wheat are China, India, United States of America, Russian Federation and France. In 2010, China was the world leader, producing 115 million metric tons of wheat. India was second in production with 80 million metric tons, followed by the U.S. at 60 million metric tons, the Russian Federation at 41 million and France at 40 million (FAOSTAT 2013)**.** In the U.S., wheat is ranked third in acreage planted and gross farm receipts behind both corn (*Zea mays*) and soybean (*Glycine max*) (USDA 2012).

Wheat has two types of growth habits, classified as winter wheat and spring wheat, with five major classes that include; hard red winter, hard red spring, soft red winter, white and durum. Hard red winter wheat is grown in the Great Plains, accounts for 40% of overall production and is primarily used for producing flour used to make bread. Hard red spring is grown in the Northern Plains and is responsible for 20% of overall production. Soft red winter wheat is predominantly grown in states that are along the Mississippi River and accounts for

20% of overall production. Soft red winter wheat is primarily used for cookies, cakes and crackers. White wheat is primarily grown in the northwestern region of the U.S., Michigan and New York, accounting for 15% of overall U.S. production. Durum wheat, used for making pasta, accounts for the lowest overall production at 5% and is grown in Montana and North Dakota (USDA 2012).

Determination of yield in wheat

Improving grain yield and yield stability across locations is the primary focus for most wheat breeding programs, and in the discipline of plant breeding, a cultivar that combines high yield and yield stability remains an elusive phenotype. The reason: yield is a quantitative trait, resulting from the cumulative expression and interaction of many genes that are strongly influenced by the environment. Yield under stress is even more complex, as environmental factors such as water availability and high temperatures can affect many physiological and biochemical processes over the seasonal life-cycle.

Grain yield in wheat is determined by a combination of different yield components. Yield components that are commonly measured include kernel weight spike^{-1}, kernels spike^{-1}, 1000 kernel weight, spike density and biomass, which includes the weight of both the grain and straw. Methods to determine yield components in wheat are fairly simple and involve sub-sampling within plots prior to harvesting for total yield (Pask et al. 2012). For example, taking a sub sample of 50 spike-bearing culms from a yield plot allows for determination of yield components either directly (i.e. weight spike^{-1}, kernels spike^{-1}) or through formulae (i.e. spike density) (Table 1). While all yield components are important, kernels m^{-2}, influenced by both spike density and

kernels spike^{-1}, consistently has the greatest contribution to total yield (Lopes and Reynolds 2012; Pask et al. 2012). Studies also show a strong relationship between grain yield and biomass accumulation, specifically biomass at anthesis and biomass during grain-filling (Tanno et al. 1985; Turner 1997).

Under abiotic stress, high yields and yield components are dependent on adequate water use and high water use efficiency (Reynolds et al. 2011; Salekdeh et al. 2009). Water use includes adequate ground cover to protect against soil moisture loss and a healthy root system for water uptake. Water use efficiency includes optimum transpiration and photo-protection. Transpiration efficiency includes a functional stay-green mechanism through sufficient leaf and spike photosynthesis (Reynolds et al. 2009). While many traits providing adaptation to stress are cumbersome to measure (i.e. water potential using a pressure chamber) secondary traits that can be used for indirect selection. Examples include canopy temperature as an indirect measurement of water use and transpiration, carbon isotope discrimination for water-use efficiency and NDVI to measure early ground cover and biomass accumulation (Reynolds et al. 2001)

Impact of abiotic stresses on wheat yield

Impact of drought stress

Drought stress impacts an estimated 42% of the global wheat production area and is considered the largest constraint to wheat yield (Kosina et al. 2007). Drought can affect wheat in many different ways depending on the timing, duration and intensity of the stress (Van Andel and Jager 1981). Wheat under drought stress experiences increased outcrossing which leads to greater levels of pollen sterility and therefore poor grain set (Bingham 1966). Drought stress in the early

stages of development has the largest effect on grain number and is considered the greatest contributor to yield loss (Bingham 1966; Fischer and Stockman 1980; Westgate et al. 1996). Drought stress prior to anthesis can reduce ear number and number of kernels per ear due to abortion (Hassan et al. 1987; Innes and Blackwell 1981), inhibit spike development and decrease spikelet number (Ji et al. 2010).

Drought stress studies have shown wheat yield reductions ranging from 10 to 95% (Foulkes et al. 2007; Kilic and Yagbasanlar 2010; Ozturk and Aydin 2004; Sieling et al. 1994). Foulkes et al. (2007) reported an average yield reduction of 2.62 tons hectare^{-1} when drought occurred at flag leaf emergence. Ozturk and Aydin (2004) examined drought stress at mid-vegetative stage, mid grain-filling stage and for an entire season and reported yield losses of 24, 40.6, and 65.6%, respectively. Biomass reductions of 40% to 60% have also been reported due to decreased growth rate resulting from drought (Lopes and Reynolds 2011; Villegas et al. 2001).

Impact of heat stress

Up to 57% of the global wheat production area will experience heat stress during the growing season, indicating heat to be one of the most important constraints to wheat yield (Kosina et al. 2007). High temperatures near anthesis generally lead to grain abortion, reducing the number of grains per spike, resulting in lower yields as a result of sink limitations (Saini and Aspinall 1982; Tashiro and Wardlaw 1990; Wheeler et al. 1996). High temperatures after anthesis decrease the rate of grain-filling (Alkhatib and Paulsen 1984; Randall and Moss 1990). Reduced root biomass can result from high temperatures after anthesis due to rapid senescence and increased respiration which affects the remobilization of assimilates (Ferris et al. 1998). With as few as

four days of heat stress, up to a 23% reduction in grain yield has been reported (Hawker and Jenner 1993; Stone and Nicolas 1994). Spiertz et al. (2006) used a three day heat shock treatment at 38/20°C during grain-fill to evaluate three genotypes grown at 18/13°C and 25/20°C (day/night). The heat sensitive genotype had a decrease in grain number per spike by 35% compared to the lower growing temperature, with a total yield reduction of 47%. Blum et al. (2001) compared yield of a RIL population between heat stressed conditions and normal winter growing conditions and reported a 47% reduction in yield in the hot environment.

Impact of soil waterlogging

Soil waterlogging affects up to 10% of global land area, including 12% of cultivated land in the United States (Boyer 1982; Setter and Waters 2003). Waterlogging is a primary constraint to wheat production in the soft red winter wheat growing region of the southeastern United States, including in the state of Arkansas, where moderate waterlogging during stand establishment occurs on a yearly basis and yield losses up to 33% have been estimated (Mason, Personal Communication). Waterlogging affects plants by decreasing O_2 availability and ceasing aerobic respiration that eventually leads to death of the plant (Gibbs and Greenway 2003). Under waterlogging conditions, Musgrave and Ding (1998) and Collaku and Harrison (2002) reported reductions in yield up to 40%, with tiller number and kernel number the yield components being most affected. Araki et al. (2012) examined waterlogging at jointing, after anthesis and at both growth stages and reported yield reductions of 10%, 15% and 35%, respectively, with 1000 kernel weight being most affected. Yaduvanshi et al. (2012) examined a waterlogging tolerant cultivar (KRL 3-4) and a waterlogging susceptible cultivar (HD) under two different pH levels.

At a pH of 8.5, the researchers reported a yield loss of 6% for KRL 3-4 and 14% for HD. At the higher pH of 9.2, a 14% yield loss was reported for KRL 3-4 and a 33% loss for HD.

Historical improvement in yield

Direct selection for yield by breeders combined with the integration of novel genes for pest resistance and phenology have historically been the major sources of yield gains in wheat (Graybosch and Peterson 2010). The relationship between date of cultivar release and yield is positive, although the rate of gain (slope) varies across different wheat germplasm pools and breeding programs (Graybosch and Peterson 2010; Green et al. 2012; Lopes et al. 2012; Sayre et al. 1997) (Table 2). The Global Wheat Breeding program at the International Maize and Wheat Improvement Center (CIMMYT) has targeted wide adaptation using a combination of shuttle breeding between its southern and northern sites, yield testing under controlled field environments, followed by international testing of elite breeding material. This system has allowed the Global Wheat Program to target mega-environments and develop spring wheat varieties with wide adaptation and tolerance to abiotic stresses (Braun et al. 1996). An analysis of 17 years of data from the CIMMYT Semi-Arid Wheat Yield Trials (SAWYT) showed gains in yield of 1.0% yr^{-1}. This was further broken down into gains of 0.7% yr^{-1} in low-yielding environments versus 1.3% yr^{-1} in high yield environments (Manes et al. 2012). While yield between high and low yielding sites were linearly related, this relationship was relatively weak (R^2=0.27). Lopes et al. (2012) reported a similar trend evaluating 30 historic CIMMYT spring wheat varieties, with a 0.9% yr^{-1} gain in high yield environments compared to only 0.5% yr^{-1} for low yielding sites. Sanchez-Garcia et al. (2013) showed that yield of 26 historic Spanish wheat

lines grown in eight environments was positively correlated with average minimum daily temperature, emphasizing the need for improving tolerance to above optimal temperatures, especially for a heat-sensitive, cool-season grasses such as wheat.

In winter wheat, Graybosch and Peterson (2010) reported no significant genetic gain in yield from 1984-2008 in the hard red winter wheat region of the Southern Great Plains of the U.S, an area which experiences significant drought and heat stress on an annual basis. This is in contrast to the Northern Great Plains where a significant increase was observed over the same time period, including an increase in the productivity of the long-term check cultivar Kharkof. The result of a more favorable wheat growing environment including increased moisture availability, milder winters and a longer growing season. Yield components associated with yield improvement over time included kernels m^{-2}, spike density and harvest index, which are likely influenced by shorter stature resulting from introgression of dwarf alleles and earlier anthesis as an abiotic stress avoidance mechanism (Table 2). Physiological traits including cooler canopy temperatures, stay-green and increased NDVI were also associated with higher yields in modern cultivars. While these studies collectively show that continuous progress has been made, with the exception of hard winter wheat, it also points to the need to continue to push the yield potential barriers and do so with an emphasis on improving adaptation and yield in marginal environments.

Spectral reflectance as a tool to determine wheat performance

Phenotyping of wheat breeding populations can be labor intensive, time consuming and is a major limiting factor in the improvement of wheat varieties. An alternative to direct selection for

yield is indirect selection using secondary traits. To be of value, a secondary trait must be correlated with a target trait, simple to measure, more heritable, or play a functional role in adaptation (Reynolds et al. 2009). Secondary traits that lend themselves to high-throughput evaluation are of particular interest, as there is a continuous need to phenotype a greater number of lines within a breeding program in order to identify new genetic diversity.

Spectral reflectance indices (SRIs) have potential as high throughput secondary measurements that are predictors of yield, biomass and other important traits. SRIs are calculated from formulas based on the absorption and reflectance of different wavelengths of light which can be measured using a spectral radiometer. SRIs have the ability to detect changes in the reflectance spectrum and translate them into changes in phenotype (Hay and Walker 1989). In agricultural research, SRIs are most commonly used for evaluating green biomass, leaf area index and green leaf area index (Reynolds et al. 2001) and have also been proposed as good estimators of total dry matter (Aparicio et al. 2002). Detection and concentration of leaf pigments can be also be estimated with SRIs, providing researchers with an indication of the plants canopy health by determining the chlorophyll to carotenoid ratio (Reynolds et al. 2001).

SRIs give researchers a phenotyping method that requires less time and labor compared to visual or mechanical selection, is non-subjective, non-destructive and allows for evaluation of a greater number of genotypes in a shorter amount of time. SRIs have the potential to serve as an aid to the "breeder's eye" and their utility has been shown across a range of environments, including both irrigated high-yield potential environments and those with abiotic stresses such as drought or heat (Busemeyer et al. 2013; Furbank 2009; Furbank and Tester 2011; Montes et al. 2007; Myles et al. 2009; White et al. 2012).

Normalized Difference Vegetative Index

Normalized Difference Vegetative Index (NDVI) is a vegetative SRI commonly used to monitor plant health and vigor. NDVI readings are influenced by two main factors: nitrogen status and biomass accumulation (Stone 1996). NDVI is calculated using the equation;

$$(NIR_{reflected} - RED_{reflected})/(NIR_{reflected} + RED_{reflected}) \quad [1]$$

where $NIR_{reflected}$ has a wavelength of 770nm and the $RED_{reflected}$ has a wavelength of 660nm. $RED_{reflected}$ is also referred to as the visible spectrum (VIS). The GreenSeeker Handheld Sensor (Optical Sensor Unit, 2002 Ntech Industries, Inc., Ukiah, CA, USA) is an active spectral radiometer that can be used to quickly and accurately measure NDVI. Leaf pigments absorb light in the photosynthetically active radiation (PAR) region (400 – 700 nm) but not in the NIR region (700 – 1200 nm) and this absorption and reflection spectrum determines the reflectance signature of the crop canopy and can be used to determine the crop's production potential. Moreover, cumulative absorption of PAR is an important parameter in determining the total amount of biomass and finishing yield (Reynolds et al. 2001). When soil nitrogen is constant across an experimental field, NDVI measurements can be interpreted a number of different ways depending on the growth stage at the time of measurement. Potential applications of NDVI include measurement of early ground cover which is of particular importance in drought stressed environments, biomass accumulation over a crop's life-cycle or during key developmental stages and as a measurement of stay-green and chlorophyll loss during grain-filling (Bennett et al. 2012b; Lopes and Reynolds 2012; Reynolds et al. 2007).

Applications of NDVI across different growing environments

Irrigated, high yielding environments

Studies have shown NDVI and related SRIs to be effective tools for predicting yield and biomass in irrigated or high-rainfall environments based on correlation analyses. Aparicio et al. (2000) studied the effectiveness of NDVI as a prediction tool and found the strongest correlation during the milky grain-fill stage for NDVI and crop dry mass ($r = 0.50$), leaf area index ($r = 0.48$) and green area index ($r = 0.40$), with the percentage of grain yield variation explained by NDVI at $R^2 = 0.40$. Similar results were observed in a follow-up study, where NDVI readings taken at booting, heading, anthesis and milk grain stages showed a positive relationship with both leaf area index ($R^2 = 0.57$ to 0.92) and total dry matter ($R^2 = 0.46$ to 0.84). In both studies, the correlation increased over-time with measurements taken in the later developmental stages showing the greatest correlations. Babar et al. (2006a) examined the utility of several spectral reflectance indices for yield prediction and selection of lines, including NDVI and two other SRIs for canopy photosynthetic area:

$$RNDVI = (R_{780} - R_{670})/ (R_{780} + R_{670}) \quad [2]$$

$$GNDVI = (R_{780} - R_{550})/(R_{780} + R_{550}) \quad [3]$$

Measurements were similar between the two photosynthetic indices at booting and heading and showed a decrease throughout the grain-filling stage. RNDVI and GNDVI were better correlated

with grain yield at the booting stage of development when compared to the other NIR-based indices, with a maximum correlation of r = 0.83. As a selection tool, NDVI measurements were able to select up to 80% of the top 20% high yielding genotypes with the best stages for genotype differentiation for grain yield at heading and during grain-filling. Similar results were reported by Prasad et al. (2007) with GNDVI able to select up to 83% of the top 25% yielding lines. A second study by Babar et al. (2007) utilizing three different populations of inbred families, reported the relationship between RNDVI and yield to range from R^2 = 0.38 to 0.68 and GNDVI and yield from R^2 = 0.57 to 0.78, depending on the population, which indicated that genetic background may influence its applicability. Broad sense heritability estimates for the SRIs across years were moderate to high (H^2 = 0.39 to 0.82) with both indices showing similar levels of heritability. It was concluded that SRIs could predict yield with a relatively high accuracy, and it could be utilized as a potential tool for selection. Other studies have reported maximum associations between NDVI and yield of R^2 = 0.44 (Reynolds et al. 1999), R^2 = 0.50 (Gutierrez-Rodriguez et al. 2004), and R^2 = 0.57 (Freeman et al. 2003). Freeman et al. (2003) reported a maximum association of R^2 = 0.83 with later season NDVI measurements. A recent study by Erdle et al. (2013) also reported a strong association between NDVI taken four times during grain-filling and grain yield across all growth stages (R^2 = 0.72 to 0.79) supporting earlier research stating that NDVI measurements in the latter growth stages are more associated with grain yield.

Application of NDVI in drought stressed environments

Gutierrez-Rodriguez et al. (2004) reported a strong association between NDVI and yield under drought conditions (r = 0.54) as did Reynolds et al. (2007), who used principle component analysis to show strong associations between NDVI and yield, with a genetic correlation of r = 0.43. Comparing trait expression of NDVI in the highest yielding lines to the yield of the lines expressing the highest level of the trait, Reynolds et al. (2007) extrapolated the theoretical yield gain from high expression of NDVI to be 3%. Babar et al. (2006b) subjected 30 CIMMYT spring wheat genotypes to varying levels of irrigation, including a one-irrigation scheme with 80 mm of water pre-planting and a two-irrigation scheme where 80mm of water was applied before planting and at booting. Results showed that there was significant genotypic variation for all spectral reflectance indices in the drought treatment and that overall NDVI and SR values were higher for the two-irrigated compared to the one-irrigated experiment. Measurements of NDVI showed a strong correlation between the booting and heading stages of growth due to the maintenance of green leaf area. The research showed a significant phenotypic correlation between NDVI measurements taken at grain-filling and grain yield with moderate to high heritability (H^2 = 0.55 to 0.92) for the different indices, indicating the measurements to be highly repeatable. The study also indicated that spectral reflectance indices have the capability of achieving genetic gain for yield and are more effective in moderate yielding environments under reduced irrigation.

Application of NDVI in heat stressed environments

Reynolds et al. (2007) observed NDVI to be strongly associated with both biomass and yield under heat stress, with a linear relationship and a maximum $R^2 = 0.54$. Estimated genetic gains from expression of NDVI under heat stress was 7%, the highest of any physiological trait evaluated with the exception of stem soluble carbohydrates. Gutierrez et al. (2010) reported high phenotypic correlations under high temperature stress for both RNDVI and yield (r = 0.85 to 0.86) and GNDVI and yield (r = 0.82 to 0.85). Hazratkulova et al. (2012) examined the effect of heat stress over two growing seasons and saw a significant negative correlation (r = - 0.80) between grain yield and the reduction of NDVI from booting to dough stage. They determined that every 1% decline in NDVI from booting to dough resulted in a 70 kg ha^{-1} grain reduction. Lopes and Reynolds (2012) used NDVI to evaluate stay-green and senescence in both a diverse population of 294 genotypes and a RIL population of 169 lines under varying levels of water and heat stress, including a full irrigation (> 500 mm of water), a terminal drought (< 200 mm of water), a high temperature/full irrigation (> 600 mm of water) and a high temperature/no irrigation (<400 mm of water). NDVI measurements taken at maturity were used as a proxy for stay-green and were found to be moderately correlated with yield in both the diverse lines (r = 0.36) and the RILs (r = 0.40) in the high temperature and high temperature/no irrigation treatments. In the same environments, rate of senescence estimated by the slope of NDVI decline over-time was lowly but significantly correlated with yield in the diverse lines (r = 0.21) but not in the RILs. Despite the low correlations, this study supports the possibility of NDVI being used as a selection tool for environments not only under irrigated and rain fed conditions but environments under abiotic stress as well.

QTL associated with yield and spectral reflectance indices

The main objective of plant breeding is to genetically improve target traits, and the use of molecular biology and genomics can aid in this process. Identifying the genomic regions associated with a trait of interest allows for trait selection through marker-assisted breeding, regardless of environment or phenotypic expression of the trait. Areas of the genome controlling quantitative traits are known as quantitative trait loci (QTL). The location of a QTL is determined by statistically associating a phenotype with allelic variation in a molecular marker (Röder et al. 1998). Molecular markers serve as flags within the genome which are linked to target genes of interest and are the result of DNA mutations such as insertions, deletions and errors in replication (Collard et al. 2005; Paterson and Wing 1993). While microsatellite markers (also known as simple sequence repeats) have historically been the most widely utilized in wheat research (Kalia et al. 2011), next generation sequencing technologies have now made single nucleotide polymorphism (SNP) markers the marker of choice for wheat research due to their abundance throughout the genome and there applicability to high throughput methods of analysis (Cavanagh et al. 2013; Poland et al. 2012). In the end, molecular markers linked to QTL serve as another tool that breeders can utilize for genetic improvement.

Studies identifying QTL for yield and yield components are numerous in the literature. A meta-analysis by Zhang et al. (2010) combined data from 59 individual QTL studies and identified 55 meta-QTL for yield related traits distributed throughout the wheat genome. Studies that have utilized regional testing for QTL identification are few and mostly limited to wide testing in the wheat growing regions of Australia (Bennett et al. 2012a; Kuchel et al. 2007; Mathews et al. 2008; McIntyre et al. 2010; Rebetzke et al. 2008). Other regional QTL studies

include winter wheat in France (Groos et al. 2003), spring wheat in the United Kingdom (Quarrie et al. 2005), and Mediterranean durum wheat (Maccaferri et al. 2008).

Few studies have identified QTL related to spectral reflectance indices and NDVI or determined their association with QTL for yield and biomass. Pinto et al. (2010) evaluated a population of 167 recombinant inbred lines (RILs) derived from two elite spring wheat cultivars, 'SeriM82' and 'Babax' in six total environments, including two drought stress (DRT), two late sown irrigated heat stress (HOT) and two fully irrigated (IRR) trials. Water ranged from 195 to 260 mm for the DRT experiments and irrigation was applied for the HOT and IRR experiments when the soil reached 50% available moisture in the top 1 m of soil. This study was significant as the RIL population was reported to have a relatively narrow range of height (~ 20 cm) and flowering time (~ 10-15 days) between RILs to minimize the pleiotropic effects of differences in phenology on trait measurement and QTL discovery. NDVI measurements were taken at the vegetative (NDVIv) and the grain-filling (NDVIg) stages using a Greenseeker Handheld Sensor. NDVIv correlated with yield in all environments, with the strongest associations observed under heat and drought stress. However, NDVIv also had a low heritability ($H^2 = 0.29$) across environments and years. A QTL for NDVIv detected on chromosome 1B explained as much as 24% of phenotypic variation and co-localized with QTL for grain number, canopy temperature and water soluble carbohydrates. Two additional NDVIv QTL were detected with minor effects, located on chromosomes 4A and 7B, with the QTL on 7B co-localizing with a QTL for maturity. QTLs for NDVIg were identified on chromosomes 2B, 4A and 7B, with co-localization between NDVIg and total grain weight detected on 2B. The authors concluded that these co-localizations could provide new possibilities for elite cultivar selection via markers (Pinto et al. 2010).

Bennett et al. (2012b) used NDVI readings at 25 days after emergence as a measurement of early vigor in drought and heat stressed environments to detect QTL in a population of 255 double haploids. In the heat stress treatment, a QTL for NDVI was detected on the short arm of chromosome 3B and co-localized with QTL for thousand kernel weight and yield. The yield QTL on this chromosome accounted for 36% of the phenotypic variation, a significant R^2 for a quantitative trait. Additional QTLs for NDVI were detected on chromosomes 1B, 2B, 3D, 4A, 5B and 7A, all of which showed co-localization with QTL for total grain yield, indicating a strong genetic association between NDVI and yield particularly under conditions of abiotic stress.

JUSTIFICATION OF THE CURRENT PROJECT

Currently, there are no reported yield QTL from germplasm adapted to the southeastern United States where soft red winter wheat is grown. This lack of knowledge is a constraint to the wheat breeders in this region. The RIL population being used in the proposed study is a cross between two elite cultivars from two different breeding programs, both of which are well adapted to the growing conditions in the southeastern U.S. Evaluating the RIL population using regional testing locations will allow for the detection of QTL associated with yield adaptation and dissection of the QTL regions into the important yield components and biomass related traits. With the exception of the studies by Bennett et al. (2012b) and Pinto et al. (2010), very little is known about the genetic control of NDVI or the genetic association of NDVI with yield and biomass. Therefore repeated measures of NDVI were used to determine the genetic control of NDVI and its relationship with grain yield and biomass.

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Table 1. Yield component calculations from a sub-sample of 50 spikes from ground level

Yield Component	**Calculation**
Total Grain Yield	Combine harvesting of whole plots
Kernel weight per spike (g)	50 Spike Grain Weight/50
1000 kernel weight (g)	The weight of 1000 kernels
Kernel number per spike (g)	(Kernel Weight per Spike)/(1000 Kernel Weight/1000)
Spike Density (spikes m^{-2})	Grain yield (g m^{-2})/Kernel weight per spike
Kernel number m^{-2}	Spike density x (kernel number per spike)
Total biomass (g m^{-2})	Spike Density x (kernel weight per spike)
Straw Biomass (g m^{-2})	Spike Density x (50 Spike Weight – 50 Spike Grain Weight)
Harvest Index (%)	50 Spike Grain Weight/50 Spike Weight

Table 2 Studies evaluating gains in yield over-time using historical sets of wheat germplasm

Germplasm Pool	No. of cultivars	Years of cultivars	Yield gain yr^{-1} Overall	Yield gain yr^{-1} High yield	%Yield gain yr^{-1} Low yield	Traits associated with yield gain	Reference
Spring	8	1962-1988	0.9%	NR	NR	Kernels m^{-2}, harvest index	Sayre et al. (1997)
Spring	566	1994-2010	1.0%	1.3%	0.7%	NR	Manes et al. (2012)
Spring	26	1997-2008	1.6%	0.9%	0.5%	Earliness, cool canopy, stay-green	Lopes et al. (2012)
Spring	26	1930-2000	0.9%	0.9%	0.7%	Short, harvest index, kernel m^{-2}	Sanchez-Garcia et al. (2013)
Hard Winter	NR	1959-2008	1.1%	NR	NR	Short, earliness	Graybosch and Peterson (2010)
Hard Winter	NR	1959-2008	0.8%	NR	NR	Short, earliness	Graybosch and Peterson (2010)
Soft Winter	50	1950-2009	0.9%	1.3%	0.6%	Spikes m^{-2}, lodging, earliness, NDVI	Green et al. (2012)[1]

CHAPTER II

Genetic Mapping of Yield and Test Weight Study

ABSTRACT

Currently, there are no reports of QTL associated with grain yield in United States soft red winter wheat (SRWW) and this lack of knowledge is a constraint to breeders in this region. The aim of this study was to identify QTL associated with grain yield, test weight and related traits to better understand the genetic control of adaptation of soft wheat to the southeastern U.S. A recombinant inbred line (RIL) population consisting of 157 lines derived from a cross between two elite cultivars ('Pioneer 26R61' and 'AGS2000') was grown from 2011-2014 in a total of 12 site-years. Overall, AGS2000 was the higher yielding parental line, out-performing Pioneer 26R61 in seven of the 12 site-years. Mean grain yield for the RILs ranged from 339 g m^{-2} to 716 g m^{-2} and test weight from 69 kg hl^{-1} to 80 kg hl^{-1}, with significant genotype, environment and genotype by environmental interaction detected for both traits. Composite interval mapping detected a total of 42 QTLs for yield, test weight, heading date and height with the phenotypic variation explained by these QTL ranging from 1.7 to 29.5%. Eleven QTL were identified for yield, with individual QTL detected in a maximum of five individual environments. Yield QTL explaining the most variance were located on chromosome 5B and were also associated with the favorable allele from AGS2000 for early heading. Eight QTL were identified for test weight, with the largest effect locus on 5D explaining 7.1% of the phenotypic variance. Co-localization between QTL for yield and test weight was identified in only one region, chromosome 5BL, indicating these two traits to be controlled by independent loci in this population. Overall, high-yielding RILs were observed to accumulate up to nine of the favorable alleles for detected yield QTL compared to low-yielding genotypes.

INTRODUCTION

Wheat is cultivated on 17% of crop land globally and is a staple food contributing more than 20% of total calories consumed (http://faostat3.fao.org). Being the most widely cultivated crop, wheat must be adapted to a range of environments (Spiertz et al. 2006). Abiotic stresses including high temperature, drought and waterlogging are a primary concern and have been reported to decrease yield up to 47% (Blum et al. 2001), 66% (Lopes and Reynolds 2011) and 40% (Araki et al. 2012), respectively. Variable climatic conditions and resulting stresses lead to unpredictable crop performance, particularly for grain yield, which has a high level of genotype by environmental interaction that makes selection for yield stability extremely difficult. Like yield, test weight (kg hl^{-1}) is another economically important trait in wheat and is commonly used for determining milling quality as it is a good indicator of flour extraction (Lyford et al. 2005). Test weight is a density measurement of seed mass per unit volume basis and, like grain yield, is difficult to select for due to quantitative inheritance and a high level of genotype by environmental interaction.

Predicted climate change and global population increases have made increasing crop yields a priority for plant breeders (Challinor et al. 2014; Tai et al. 2014). Direct selection for yield by breeders combined with the integration of novel genes for pest resistance and phenology have historically been the major sources of genetic yield gains in wheat (Graybosch and Peterson 2010). In spring wheat, historical yield gains of 1.0% yr^{-1}have been reported, including 0.7% yr^{-1} in low-yielding environments versus 1.3% yr^{-1} in high yield environments (Manes et al. 2012). In winter wheat, Graybosch and Peterson (2010) reported no significant genetic gain in yield from 1984-2008 in the hard red winter wheat region of the Southern Great Plains of the U.S, an

area which experiences significant drought and heat stress on an annual basis. This is in contrast to the Northern Great Plains where a significant increase in grain yield was observed over the same time period, including an increase in the productivity of the long-term check cultivar Kharkof, the result of a more favorable wheat growing environment including increased moisture availability, milder winters and a longer growing season.

Genetic and genomic tools assist breeders in selection of superior breeding lines. Genetic mapping can be used to identify genomic regions associated with traits of interest, such as yield, which can lead to development of markers linked to promising quantitative trait loci (QTL) that can be used for marker-assisted breeding. Studies identifying QTL for yield and yield components are numerous in the literature. A meta-analysis by Zhang et al. (2010) identified 55 meta-QTL for yield-related traits in wheat which combined data from 59 individual QTL studies. Likewise, Acuña-Galindo et al. (2014) identified meta-QTL associated with yield and heat and drought-adaptive traits. Studies which have utilized testing across a broad region for QTL detection (the aim of this research) are few and limited to the spring wheat growing regions of Australia (Bennett et al. 2012a; Kuchel et al. 2007a; Mathews et al. 2008; McIntyre et al. 2010; Rebetzke et al. 2008), winter wheat in France (Groos et al. 2003), spring wheat in the United Kingdom (Quarrie et al. 2005), and Mediterranean durum wheat (Maccaferri et al. 2008). To date, there are no reports of QTL associated with grain yield in U.S soft red winter wheat (SRWW), which constitutes 4.06 million hectares (10.044 million acres) cultivated annually (http://www.ers.usda.gov/data-products/wheat-data.aspx).

Therefore, the goal of this study was to carry out a robust QTL analysis utilizing an elite recombinant inbred line population and key field testing locations throughout the southern U.S.

By utilizing a high density genetic map containing 2,734 markers, tightly linked marker-trait associations will be identified that can be utilized by breeders for genetic improvement.

MATERIALS AND METHODS

Plant Material

A recombinant inbred line (RIL) population consisting of 178 lines was used for this study. The RIL population was derived from a cross between two historically elite cultivars, 'Pioneer 26R61' and 'AGS2000', both of which are well adapted to the southeastern United States. Pioneer 26R61 (Omega 78/S76/Arthur 71/3/Stadler//Red-coat/Wisconsin 1/5/Coker 747/6/Pio. 2555 sib) was developed by Pioneer Hi-Bred and originally selected as a parental line because of its yield potential and resistance to stripe rust, leaf rust, soil borne wheat mosaic virus and Hessian fly. AGS2000 (Pio.2555/PF84301//FL 302; PI 612956) was developed by the University of Georgia and Florida Experiment Station (Hao et al. 2011). The cultivars have historically been used as yield checks in the Uniform Southern Soft Red Winter Wheat Nurseries (USSRWWN) and the development of the RIL population (referred to herein as P/A) has previously been described (Hao et al. 2011). The population segregates for genes related to photoperiod (*Ppd-D1, Ppd-B1 and Ppd-A1*) and vernalization (*vrn-A1 and vrn-B1*) and resistance to soil-borne mosaic virus (*Sbm1*). Following the 2011-2012 season, very early flowering and very late flowering lines were eliminated from further analyses to have a more narrow (~10 days) range of anthesis. The population does not segregate for any known genes controlling height (*Rht* genes) as both parents contain the *Rht-D1* dwarfing allele located on chromosome 4D.

Experimental Design for Regional Yield Testing

A summary of the testing locations is presented in Table 1. The P/A RILs were planted in five locations in the 2011-2012 growing season, six locations in the 2012-2013 growing season and three locations in the 2013-2014 growing season. Testing sites included Fayetteville, Stuttgart and Marianna, AR, Plains, GA, Farmersville, TX and Baton Rouge, LA. Mariana, AR was not used as a testing site in 2011-2012, and only the Arkansas locations were sown in 2013-2014. Trials in Baton Rouge, LA were not harvested in 2011-2012 due to severe lodging resulting from a late freeze. In 2011-2012 and 2012-2013 a randomized complete block design with two replications was used for all locations. Both parental cultivars (Pioneer 26R61 and AGS2000) and AGS2035, a direct selection out of the P/A population were used as repeated checks every 20 plots. In 2013-2014, a Type-I augmented design with two replications was used which consisted of eight incomplete blocks with the two parents used as replicated checks.

Plots were 1.5 m wide by 4.5 m long consisting of 7 rows spaced 7 inches apart. All trials all years were drill seeded at a rate of approximately 118 kg of seed hectare^{-1}. Planting and harvest dates and trial management varied based on recommendations at each location. Both Fayetteville and Stuttgart received a single fungicide application of 50 mL Tilt® (Syngenta Group Company) ha^{-1} to control for stripe rust.

Trait measurement

Grain yield (g m^{-2}) was determined at all locations on whole plots by combine harvesting and adjusting yield to 13% moisture. Plant height was measured at maturity from the soil surface to the top of each plot, excluding awns. Days-to-heading was measured in Julian days when 50% spike emergence for each plot. Test weight was determined on a volume basis either manually or using a GAC2500AGRI DICKEY-john Grain Moisture Tester (Churchill Industries, Minneapolis, MN). Climatic data was collected by individual experiment stations and checked alongside the National Weather Service Weather Forecast Office (www.srh.noaa.gov) and Weather Underground (http://www.wunderground.com).

Statistical analyses

Statistical analyses were conducted using various procedures in SAS 9.3 (SAS Institute Inc. 2011, Cary, NC). Means were calculated for individual site-years for parental lines and RILs using PROC MEAN. Analysis of variance and significance testing were performed within individual site-years as well as across all site years using PROC MIXED with genotype as a fixed effect. A separate analyses was preformed to calculated broad-sense heritability of measured traits from the variance components using TYPE3 sum of squares with all effects treated as random and using the following formula: $\sigma^2_G / (\sigma^2_G + \sigma^2_{GEI}/e + \sigma^2_E/re)$, where σ^2_G, σ^2_{GEI}, σ^2_E are genotypic variance, genotype-by-environmental variance and error variance, respectively, and e and r are the numbers of environments and number of replications. The site regression analysis (SREG) was performed to better understand genotype and the genotype by

environment interaction effects with graphics created using PROC TREE and PROC CLUSTER (Crossa and Cornelius 1997).

Genetic Map Construction

A genetic linkage map for the P26R61/AGS2000 population was previously developed by Hao et al. (2011). The original published linkage map contained 895 total loci spanning 2,659 centimorgans and included 747 diversity array technology (DArT) markers, 146 simple sequence repeats (SSR), and two powdery mildew resistance genes; Pm3-STS and Pm3a. Since the original published map, an additional 1,839 single nucleotide polymorphism (SNP) markers derived from the 9K Infinium SNP Array (Illumina Inc. San Diego, CA) have been integrated into the genetic map, bringing the total number of markers to 2,734 (Brown-Guedira, *In-prep*). This was carried out in collaboration with the USDA-ARS Regional Genotyping Centers in Fargo, ND and Raleigh, NC. In total, there are 26 linkage groups which span all 21 wheat chromosomes.

QTL detection

Best linear unbiased predictors (BLUPs) for line and trait combinations within individual environments and for each line and trait in a combined analysis using all environments were used for QTL analysis in WINQTL Cartographer version 2.5 (Wang et al. 2007). For QTL detection, single marker analysis was initially used to identify genetic markers significantly associated with phenotypic traits. Composite interval mapping (CIM) with forward/backward regression was

then used to determine QTL positions at a significance threshold of $P < 0.05$ and a maximum of 25 control markers. QTL that were significant in both the combined analysis and at least two individual site-years were considered stable and reported herein.QTL were detected for total grain yield, test weight, heading date and height in order to better understand pleiotropic effects within a detected region. Linkage groups were drawn using MapChart 2.2.

RESULTS

Climatic summary and phenotypic performance

Considering the same locations across years, similar climatic conditions were generally observed (Table 2). Total rainfall among environments varied from 563.1 to 1368.3 mm throughout the growing season. As expected, environments located at lower latitudes experienced more days with temperatures greater than 30°C prior to anthesis than those located at higher latitudes. Of the site-years located at lower latitudes, three site-years experienced more than 48 days with temperatures greater than 30°C, with LA13 and TX12 recording 52 days and 60 days, respectively.

Mean grain yield for the RILs ranged from a minimum of 339 g m^{-2} in Stutt12 to a maximum 716 g m^{-2} at GA13 (Table 3). AGS2000 yielded higher than P26R61 in seven of the 12 site-years. The two largest mean grain yield differences between parental lines were both observed in the Plains, GA location. In GA12, P26R61 yielded 71 g m^{-2} higher than AGS2000 which yielded 447 g m^{-2}. The opposite was observed in GA13 as AGS2000 yielded 86 g m^{-2} higher than P26R61 (734 g m^{-2}). With the exception of Stutt12, mean grain yield of the RILs was lower than both parental lines. Test weight for the RIL population ranged from 69 kg hl^{-1} at

Fay13 to 80 kg hl^{-1} at Fay14. For all environments, P26R61 had a mean test weight higher than or equal to AGS2000 and the RIL population. The shortest average height for the RIL population and parental lines occurred in Fay14 while the tallest average height occurred in GA13. The earliest heading location was LA13 where the RIL population averaged 85 Julian days until heading. The latest heading mean of the RIL population occurred in Fay14.

Analysis of variance and correlations between environments and traits

Differences across environments explained the greatest amount of variation within the RILs for all traits, although significant genotype and genotype by environmental interactions were also detected (Table 4). Broad-sense heritability ranged from moderate ($H^2 = 0.57$) for test weight to highly heritable ($H^2 = 0.92$) for heading date, with yield showing a moderate heritability ($H^2 = 0.66$) across all 12 site-years.

Site regression analysis based on grain-yield of the RILs identified four main environmental clusters (C1-C4) (Figure 1). With the exception of Mar13 and Stutt13, Arkansas locations tended to group together (C1:2), as did locations at lower latitudes. Pearson's correlation coefficients between yield and other phenotypic traits within each cluster are presented in Table 5. Heading date had a negative correlation with grain yield overall, with the highest correlation observed in C1 ($r = -0.63$, $P < 0.001$) and a non-significant correlation observed in C2. Height correlations ranged from negative in C4 ($r = – 0.18$, $P < 0.01$) to strongly positively in C1 ($r = 0.62$, $P < 0.001$). For both C1 and C2, which included all but two of the Arkansas site-years, a taller stature was associated with higher grain yield. Test weight was generally positively correlated with yield with the exception of C1 where a low but significant correlation was observed ($r = 0.07$, $P < 0.05$).

Yield and test weight QTL

QTL were detected using BLUP values calculated for individual site years and with all environments analyzed together in a combined analysis (Table 6, Figure 2). When considering only the results of the combined analysis, a total of 42 QTL were identified for the four traits reported (Table 5). For grain yield, eleven stable QTL were detected and located on chromosomes 1A, 1D, 2B, 2D, 3A, 3B, 5B, 6A and 7A. Both parents contributed favorable alleles for yield to the population, including four from AGS2000 and seven from P26R61. The AGS2000 allele at *QYld.ua-5B* explained the largest percent of the phenotypic variance for any individual QTL (R^2 = 8.9%) with an additive effect of 6.6 g m^{-2}. The P26R61 allele at *QYld.ua-2B* explained the second highest portion of phenotypic variance (R^2 = 7.4%) and an additive effect of 6.5 g m^{-2}. Three QTL were detected on chromosome 3B and together explained a total of 12.5% of the variation in grain yield. *QYld.ua-2B* and *QYld.ua-7A* were the most stable QTL being detected in five environments over the three year period. Taken together, detected yield QTL explained a total of 48.8% of the phenotypic variance in grain yield.

For test weight, eight stable QTL were detected and located on chromosomes 1A, 1B, 2D, 5B, 5D, 6B and 6DL. Of the eight QTL detected, both parents contributed four favorable alleles. The variance explained by QTL for test weight ranged from R^2 = 2.3 for *QTw.ua-6B* to R^2 = 6.7% for *QTw.ua-5D*. *QTw.ua-5D* accounted for the highest variance explaining 6.7% of the phenotypic variation. *QTw.ua-2D* was the most stable test weight QTL, detected in six site-years and explaining 5.4% of the phenotypic variation. Taken together, test weight QTL explained 31.4% of the total phenotypic variance for the trait.

Phenotypic variance explained by all height QTL totaled 63% while heading QTL totaled 70.4%. A notable QTL for heading date was detected on chromosome 7D-2 and explained 7.6% of the phenotypic variation. *QHd.ua.7D-2* was flanked by *Vrn-D3*, a known gene regulating vernalization requirement in wheat with the allele from this P26R61 delaying heading by one day. *QHd.ua-2D* explained 20.2% of the phenotypic variance in heading date and showed the most stability, being detected in eight of the twelve environments. This QTL region also co-localized with a height QTL, *QHgt.ua-2D*, accounting for 29.5% of the height variation in the RIL population. AGS2000 contributed the favorable allele for both *QHd.ua-2D* and *QHgt.ua-2D* which co-localized on marker *Ppd-D1*, a marker for the regulation of photoperiod sensitivity. This is in agreement with the phenotypic results, with AGS2000 being the earlier heading parent at all locations with the exception of Fay14 and Stutt13 in which the average heading date were the same for both parents.

Accumulation of favorable alleles

With numerous small effects QTL, the accumulation of favorable alleles at the 11 detected yield loci was examined further in both the highest and lowest yielding RILs. The top five yielding RILs across all locations were shown to accumulate six to nine of the favorable QTL alleles, compared to a maximum of three in the five lowest-yielding lines (Table 7). Furthermore, favorable alleles at *QYld.ua-2B* and *QYld.ua-5B* were observed in all five of the top-yielding RILs, with the favorable alleles contributed by opposite parents contributing to transgressive segregation within the population. This is not unexpected, as these yield QTL explained the greatest amount of the phenotypic variation for grain yield. Interestingly, the highest yielding

line, RIL-47, accumulated only six favorable alleles, which could be an indication of epistatic effects.

DISCUSSION

Phenotyping

Phenotypic variation in a mapping population, as was observed within the P/A RILs, is a key component for effective detection of meaningful QTL. Both P26R61 and AGS2000 were selected as parents due to their wide adaptation to the southeastern United States. Hao et al. (2011) described AGS2000 as the higher-yielding variety of the two parental lines and although AGS2000 was not significantly different from P26R61, it had higher grain yield in seven of the twelve site-years. Significant differences in grain yield were observed across environments and within individual locations for the RIL population (data not shown) with mean grain yield across environments ranging from 339 to 716 g m^{-2}. The RIL population mean grain yield extended beyond the range of the parental lines, indicating the possibility of transgressive segregation. The largest range in grain yield within a single environment was observed in GA13 (175-992 g m^{-2}), which was also the highest yielding location with a mean grain yield of 716 g m^{-2}. This is not unexpected, as AGS2000 was developed at the University of Georgia, is well-adapted to the region, and the 2012-2013 season had very favorable growing conditions. Comparing the 2011-2012 and 2012-2013 growing seasons, GA13 experienced a 79.4% increase in total rainfall from 563.1 to 1010.4 mm and a mean yield increase of 81%, from 396 to 716 g m^{-2}. Though LA13 recorded the largest amount of rainfall of any site-year, with 1368.3 mm, it also recorded the second largest number of days with max temperature > 30°C post anthesis, which may have

negatively impacted yield. TX12 and Stutt12 also recorded over 40 days above 30°C post anthesis. Of the 40 days above 30°C, each location recorded ten days of 35°C or more. High temperatures after anthesis can be an indication of heat stress which has been reported to reduce grain yield by 23% with as few as four days with temperatures above 35°C (Hawker and Jenner 1993; Stone and Nicolas 1994). Kuchel et al. (2007b) reported that days above 30°C accounted for as much at 49.2% of the variation of grain yield with as much as a 75.8 kg ha^{-1} reduction per day over 30°C. Potential causes of reduced grain yield due to heat stress include decreased rate of grain-filling, a reduction in root biomass due to rapid senescence and remobilization of assimilates due to increased respiration (Alkhatib and Paulsen 1984; Ferris et al. 1998; Randall and Moss 1990). As high temperatures post anthesis are common in the southeastern United States, particularly in the lower latitudes of the region, it is important to select for earlier flowering varieties. This was also indicated in the phenotypic correlation groups where significant negative correlations were observed between yield and heading date within C1, C3 and C4.

Impact of genotype x environment interaction on QTL detection

Genotype by environmental interaction has a strong influence on QTL detection when studies are carried out across variable environments and years. Considering yield, detected QTL were only found to be stable in a maximum of five environments (Table 6), due to both the quantitative inheritance of yield (Table 7) and influence of the environment. An example of this was seen for *QYld.ua-1A*, which was only detected in GA12 and Stutt13, where similar yield performance for the RILs and a similar a number of days above 30°C (31 and 32 days, respectively) was observed in both environments. Similarly, *Yld.ua-3B.1* was detected in both TX12 and LA13, both lower

latitude locations that experienced 60 and 52 days with maximum temperature above 30°C, respectively, the most of any locations. *QYld.ua-3B.1* could be especially important to the improvement of grain yield in heat stress, lower latitude environments. *QTw.ua-5B* was detected in both GA13 and LA13 which were both high yielding environments that accumulated the largest amount of rainfall over the growing season with 1010.7 and 1368.3 mm, respectively. *QTw.ua-5B* therefor could be important for the improvement of test weight in high rainfall or irrigated environments. Though the overall goal is to determine yield and test weight QTL viable for wheat improvement across the southeastern U.S., results of this study suggest it may also be important to utilize more "environment specific" QTL.

Pleiotropic QTL

Co-localization between yield and either heading or height QTL occurred on chromosomes 2D and 5B. On chromosome 2D, QTL for yield (*QYld.ua-2D*) and heading date (*QHd.ua-2D.2*) co-localizing together, with the P26R61 allele contributing to higher yield and a later heading date. This genomic region has also been reported in past research as a region of interest for marker assisted selection (Kumar et al. 2007; Maphosa et al. 2014; Zhang et al. 2010). The greatest number of yield QTL were observed on Chromosome 3B with one co-localizing with a heading date QTL, with *QYld.ua-3B.2* explaining 6.6% other the variation in grain yield. Bennett et al. (2012b) also reported chromosome 3B as a significant chromosome influencing grain yield, kernel m^{-2}, thousand kernel weight, and canopy temperature at grainfill. *QYld.ua-5B* explained the most phenotypic variance in grain yield of all detected QTL at 8.4%. *QYld.ua-5B* also co-localized with QTL for test weight (*QTw.ua-5B*) and heading date (*QHd.ua-5B.1*). AGS2000 contributed the favorable allele for both *QYld.ua-5B* and *QTw.ua-5B* while P2R61 contributed

the favorable allele for *QHd.ua-5B.1* contributing to earlier heading. This highlights an interesting relationship where higher yield and test weight were correlated with an earlier heading date. This allelic regions could prove to be useful in the improvement of wheat yield as earlier flowering time has been shown to be beneficial for avoidance of abiotic stresses and better adaption for a variety of environments (Jung and Muller 2009; Poland et al. 2009).

Independent Yield QTL

In a QTL analysis it is important to not only consider genomic regions associated with multiple traits of interest and how they influence each other, but also independent QTL effects. Of the eleven QTL detected for yield, six were found independent of QTL for phenological traits such as heading or height. Comparing to previously published QTL results, the region of *QYld.ua-3A* located on chromosome 3A has been the most commonly reported, but explained only 3.0% of the phenotypic variation in this study (Bennett et al. 2012b; Campbell et al. 2003; Mengistu et al. 2012). Similarly, genomic regions on chromosome 3B have been previously reported to have a significant impact on total grain yield (Bennett et al. 2012b; Kumar et al. 2007; Zhang et al. 2010). In this study, *QYld.ua-3B.1* and *QYld.ua-3B.3* explained a total of 5.8% of phenotypic variation. Maphosa et al. (2014) also reported a yield QTL on chromosome 6A, as was reported here for *QYld.ua-6A,* which explained 5.4% of the phenotypic variation for yield. While the importance of the detected chromosomes can be confirmed, the exact relationship between the QTL reported here and in previous studies remains unclear due to the lack of common makers across genetic maps,

With the exception of *QTw.ua-5B* and *QTw.ua-6B.2,* all test weight QTL were detected independent of other measured traits, in agreement with previous research showing independent

inheritance of test weight (Bennett et al. 2012a; Groos et al. 2003). *QTw.ua-5D* accounted for the largest amount of phenotypic variance of all test weight QTL. McCartney et al. (2005) reported a QTL for test weight on chromosome 5D that was 50cM distal to *QTw.ua-5D* that co-localized with marker *Xwmc765* and explained 7.1% of the phenotypic variance. *QTw.ua-5D* is also 50cM distal to a test weight QTL reported by Sun et al. (2009) co-localizing on a common maker, *Xgwm174*.

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Table 1. Description of testing locations during the duration of the study

Location	No. of Years	Latitude, Longitude	Soil-series	Taxonomic Class
Fayetteville, AR	3	36.08, -94.16	Captina Silt Loam	Fine-silty, siliceous, active, mesic Typic Fragiudults
Marianna, AR	2	34.77, -90.76	Loring silt loam	Fine-silty, mixed, active, thermic Oxyaquic Fragiudalfs
Stuttgart, AR	2	34.5, -91.55	Dewitt silt loam	Fine, smectitic, thermic Typic Albaqualfs
Farmersville, TX	2	33.36, -96.37	Houston black clay	Fine, smectitic, thermic Udic Haplusterts
Baton Rouge, LA	1	30.45, -91.14	Thibaut silty clay	Clayey over loamy, smectitic over mixed, superactive, nonacid, hyperthermic Vertic Epiaquepts
Plains, GA	2	32.03, -84.39	Greenville sandy loam	Fine, kaolinitic, thermic Rhodic Kandiudults

Table 2. Summary of climatic data across 12 site-years

	Average Min Temp (°C)		Average Max Temp (°C)		Rainfall (mm)		Days Max Temp > 30°C	
Site Year (Code)	Pre Anthesis	Post Anthesis	Pre Anthesis	Post Anthesis	Pre Anthesis	Post Anthesis	Pre Anthesis	Post Anthesis
Baton Rouge 12-13 (LA13)	7.9	17.3	20.9	28.6	867.9	500.4	5	47
Farmersville 11-12 (TX12)	6.9	18.4	18.7	30.2	533.4	107.7	8	52
Farmersville 12-13 (TX13)	5.4	15.7	18	27.5	396.2	218.4	3	34
Fayetteville 11-12 (Fay12)	2.8	14	15.3	27.6	555.8	136.9	0	34
Fayetteville 12-13 (Fay13)	0.4	11.8	13.8	24.3	409.7	370.3	0	19
Fayetteville 13-14 (Fay 14)	-1.8	12.3	11.8	24.4	508.5	268.0	0	12
Marianna 12-13 (Mar13)	4.1	14.7	14.2	25.7	630.2	351.8	0	31
Marianna 13-14 (Mar14)	2.3	15.4	12.9	26.0	467.6	437.4	3	35
Plains 11-12 (GA12)	6.3	14.6	20.2	28.1	382.8	180.3	2	29
Plains 12-13 (GA13)	5.1	14.6	18.7	26.4	660.1	350.5	1	23
Stuttgart 11-12 (Stutt12)	6.9	17.5	17.2	28.7	529.8	149.4	4	44
Stuttgart 12-13 (Stutt13)	4.7	15.7	14.5	26.1	497.8	283.5	0	32

[a]Min indicates Minimum, Max indicated Maximum

Table 3. Summary for traits measured in the Pioneer 26R61 x AGS 2000 recombinant inbred lines across 12 site-years

		Yield (g m^{-2})				Test Weight (kg hl^{-1})				Height (cm)				Heading Date (Julian)			
Site Year	Code	AGS 2000	Pioneer 26R61	RIL	RIL Range	AGS 2000	Pioneer 26R61	RIL	RIL Range	AGS 2000	Pioneer 26R61	RIL	RIL Range	AGS 2000	Pioneer 26R61	RIL	RIL Range
Baton Rouge 12-13	LA13	488	447	440	295 - 627	70	72	70	46 - 75	-	-	-	-	83	89	85	70 - 108
Farmersville 11-12	TX12	457	447	399	81 - 564	-	-	-	-	-	-	-	-	-	-	-	-
Farmersville 12-13	TX13	391	391	372	192 - 523	73	76	75	68 - 83	-	-	-	-	-	-	-	-
Fayetteville 11-12	Fay12	543	520	502	280 - 659	74	74	74	66 - 77	91	94	94	80 - 120	93	94	92	86 - 98
Fayetteville 12-13	Fay13	456	471	435	101 - 705	66	71	69	49 - 77	-	-	-	-	117	121	119	110 - 125
Fayetteville 13-14	Fay14	419	394	386	190 - 545	81	81	80	74 - 86	70	69	71	51 - 99	130	130	129	122 - 135
Marianna 12-13	Mar13	410	433	391	181 - 567	74	76	74	65 - 82	99	99	101	81 - 124	113	115	113	106 - 122
Marianna 13-14	Mar14	513	529	502	290 - 613	79	79	79	73 - 83	87	91	90	76 - 109	119	121	120	114 - 127
Plains 11-12	GA12	447	518	396	94 - 665	-	-	-	-	-	-	-	-	-	-	-	-
Plains 12-13	GA13	820	734	716	175 - 992	76	78	75	54 - 79	102	108	105	86 - 124	94	101	96	85 - 117
Stuttgart 11-12	Stutt12	373	336	339	165 - 477	78	78	76	50 - 87	73	75	76	55 - 91	-	-	-	-
Stuttgart 12-13	Stutt13	421	399	397	193 - 557	70	73	72	63 - 79	89	88	91	73 - 109	115	115	113	107 - 121
Mean		477	475	440		74	76	74		85	87	87		105	109	107	

Table 4. Percent of total variance due to genotype, environment, genotype x environment (G X E), replication and error for the Pioneer 26R61 x AGS 2000 recombinant inbred lines.

Yield Component	Genotype (G)	Environment (E)	G X E	Rep (Loc)	Residual	H^2
Yield (g m^{-2})	0.04***	0.60***	0.15***	0.01***	0.20	0.66
Test Weight (kg hl^{-1})	0.04***	0.52***	0.15***	0.00***	0.29	0.57
Height (cm)	0.09***	0.76***	0.03**	0.02***	0.10	0.90
Heading Date (Julian)	0.04***	0.93***	0.02***	0.00***	0.01	0.92

* Significant at P = 0.01
** Significant at P = 0.001
*** Significant at P = 0.0001

Table 5. Correlation of agronomic traits with yield for the Pioneer 26R61 x AGS 2000 recombinant inbred lines within each environmental cluster

	Test weight (kg hl^{-1})	Heading (Julian)	Height (cm)
Cluster 1	- 0.07*	- 0.63***	0.62***
Cluster 2	0.10	- 0.001	0.24***
Cluster 3	0.56***	- 0.33***	- 0.10
Cluster 4	0.30***	- 0.25***	- 0.18**

* Significant at P = 0.05
** Significant at P = 0.01
*** Significant at P = 0.001

Table 6. Summary of QTL detected for the Pioneer 26R61 x AGS 2000 recombinant inbred lines across 12 site-years

Trait	Chromosome - Marker	Position	LOD	R2	Additive Effect	Source[a]	Environments
Grain yield							
QYld.ua-1A	1A - IWA6341	66.9	4.2	0.029	3.53	A	GA12, Stutt13
QYld.ua-1D	1D - IWA3549	9.8	2.6	0.018	2.84	A	Stutt12, Mar13
QYld.ua-2B	2B - wPt-741721	108.2	10.1	0.074	6.55	P	TX12, Fay13, Stutt13, GA13, Fay14
QYld.ua-2D	2D - wPt-2761, wPt-2544	54.1	3.0	0.035	4.00	P	Mar13, TX13
QYld.ua-3A	3A -IWA2397 & IWA8000	51.1	4.7	0.033	3.87	P	Stutt13, Fay14
QYld.ua-3B.1	3B - IWA4801	10.0	4.9	0.034	3.91	A	TX12, LA13
QYld.ua-3B.2	3B - Xwmc326	108.8	8.9	0.067	5.58	P	Stutt12, TX12, Stutt13
QYld.ua-3B.3	3B - wPt-6834	145.6	3.4	0.025	3.57	P	Fay12, Fay14
QYld.ua-5B	5B - Xgwm408	97.5	11.1	0.085	6.62	A	GA12, TX12, GA13, Mar14
QYld.ua-6A	6A - IWA1205, wPt-667170	14.8	6.2	0.055	4.87	P	Stutt12, GA12, Stutt13, TX13
QYld.ua-7A	7A - wPt-0494	31.9	4.9	0.034	3.98	P	Fay12, TX12, Fay13, LA13, Fay14
Test weight							
QTw.ua-1A	1A - IWA1387	21.9	2.7	0.024	0.11	A	Stutt13, Fay14
QTw.ua-1B	1B - wPt-4688	39.9	4.8	0.044	0.15	P	Fay13, Mar13, GA13, LA13, Mar14
QTw.ua-2D	2D - wPt-5586	93.2	6.0	0.054	0.17	A	Fay12, Stutt13, Mar13, TX13, Fay14, Mar14
QTw.ua-5B	5B - IWA8097	91.9	2.6	0.025	0.11	A	GA13, LA13
QTw.ua-5D	5D - Xgwm212	106.2	7.1	0.067	0.19	P	Mar13, TX13, Mar14
QTw.ua-6B.1	6B - IWA2475	15.5	5.8	0.054	0.17	P	Stutt13, LA13
QTw.ua-6B.2	6B - IWA7663	64.5	2.6	0.023	0.11	A	Fay12, LA13, Fay14
QTw.ua-6DL	6DL - wPt-3127	24.4	2.7	0.024	0.11	P	Mar13, GA13
Height							
QHgt.ua-1A	1A - IWA3434	84.0	4.0	0.023	0.61	P	Stutt13, Fay14
QHgt.ua-2A.1	2A - Xwmc667	15.4	5.8	0.031	0.73	P	Mar13, Fay14, Mar14
QHgt.ua-2A.2	2A - wPt-6139	45.4	5.8	0.031	0.74	P	Stutt13, Fay14, Mar14
QHgt.ua-2D	2D - Ppd-D1	12.0	32.6	0.295	2.27	A	Mar13, Stutt13, GA13, Fay14, Mar14
QHgt.ua-3B	3B - wPt-8238	42.1	3.8	0.020	0.61	A	Stutt13, Mar14
QHgt.ua-4B	4B - Xgwm6	24.1	7.2	0.039	0.80	A	GA13, Fay14
QHgt.ua-5B.1	5B - wPt-9006	0.0	3.1	0.018	0.55	P	Mar13, Fay14

QHgt.ua-5B.2	5B - Xbarc243	146.6	5.7	0.031	0.76	A	Stutt13, Mar13, GA13
QHgt.ua-6B	6B - Xgwm107	67.2	6.5	0.035	0.81	P	Stutt13, GA13
QHgt.ua-7D-2	7D-2 - IWA6822, Xbarc172	47.6	7.4	0.068	1.05	P	Stutt13, Mar13, GA13, Fay14, Mar14
QHgt.ua-7A	7A - wPt-6872	34.9	7.0	0.041	0.85	P	GA13, Mar14
Heading Date							
QHd.ua-2A	2A - wPt-0568	44.4	7.7	0.058	0.75	P	Fay12, Fay13, Stutt13, Mar13, GA13, LA13
QHd.ua-2D.1	2D - Ppd-D1	11.0	22.9	0.203	1.40	A	Fay12, Fay13, Stutt13, Mar13, GA13, LA13, Fay14, Mar14
QHd.ua-2D.2	2D - IWA5637	44.3	5.6	0.032	0.57	P	Fay12, Fay13, Mar13, GA13, LA13
QHd.ua-3B.1	3B - rPt-8896	60.4	3.6	0.020	0.47	A	Fay12, Stutt13, GA13
QHd.ua-3B.2	3B - Xwmc326	108.5	4.3	0.025	0.50	P	Fay12, Mar13, Mar14
QHd.ua-4B	4B - Xwmc141	68.0	4.1	0.023	0.52	A	Fay12, Fay13, LA13
QHd.ua-5AL	5AL - IWA1398	18.9	9.6	0.060	0.80	A	Fay12, Fay13, Mar13, GA13, Fay14, Mar14
QHd.ua-5B.1	5B - Xwmc75	96.4	13.9	0.093	0.98	P	Fay13, Mar13, GA13, LA13, Mar14
QHd.ua-5B.2	5B - Xbarc243	146.6	9.1	0.054	0.77	A	Mar13, GA13, LA13, Fay14, Mar14
QHd.ua-6A	6A - Xgwm617	149.0	3.2	0.019	0.44	A	Stutt13, Fay14
QHd.ua-6B	6B - wPt-732062	25.7	7.3	0.042	0.65	A	Fay13, Stutt13, GA13, LA13, Fay14, Mar14
QHd.ua-7D-2	7D-2 - wPt-733087, Vrn-D3	19.6	9.8	0.077	0.86	P	Fay12, Fay13, Mar13, Mar14, LA13

[a]P indicates favorable allele contributed by the Pioneer 26R61 parent
[a]A indicates favorable allele contributed by the AGS 2000 parent

Table 7. Allele accumulation in the highest and lowest yielding Pioneer 26R61 x AGS 2000 recombinant inbred lines

		QTL											
		1A	1D	2B	2D	3A	3B.1	3B.2	3B.3	5B	6A	7A	
	Fav. Allele	A	A	P	P	P	A	P	P	A	P	P	No. favorable alleles
Highest Yielding	RIL-47	-	-	+	+	-	+	-	+	+	-	+	6
	RIL-127	+	-	+	H	+	-	+	+	+	+	-	8
	RIL-71	+	+	+	-	+	-	+	+	+	H	+	9
	RIL-160	-	+	+	+	-	-	-	+	+	-	+	6
	RIL-9	+	-	+	+	+	+	+	-	+	H	+	9
Lowest Yielding	RIL-155	+	-	-	-	-	-	+	-	+	-	-	3
	RIL-11	-	-	-	+	+	-	-	-	+	-	-	3
	RIL-36	+	-	-	-	-	-	-	-	-	-	-	1
	RIL-175	-	+	-	+	-	+	-	-	-	-	-	3
	RIL-173	-	-	-	+	+	-	-	-	-	-	+	3

[a]A indicates allele contributed by the AGS 2000 parent
[b]P indicates allele contributed by the Pioneer 26R61 parent
[c]+ indicates RIL inherited the favorable allele
[d]- indicates RIL inherited the non-favorable allele
[e]H indicates heterozygosity

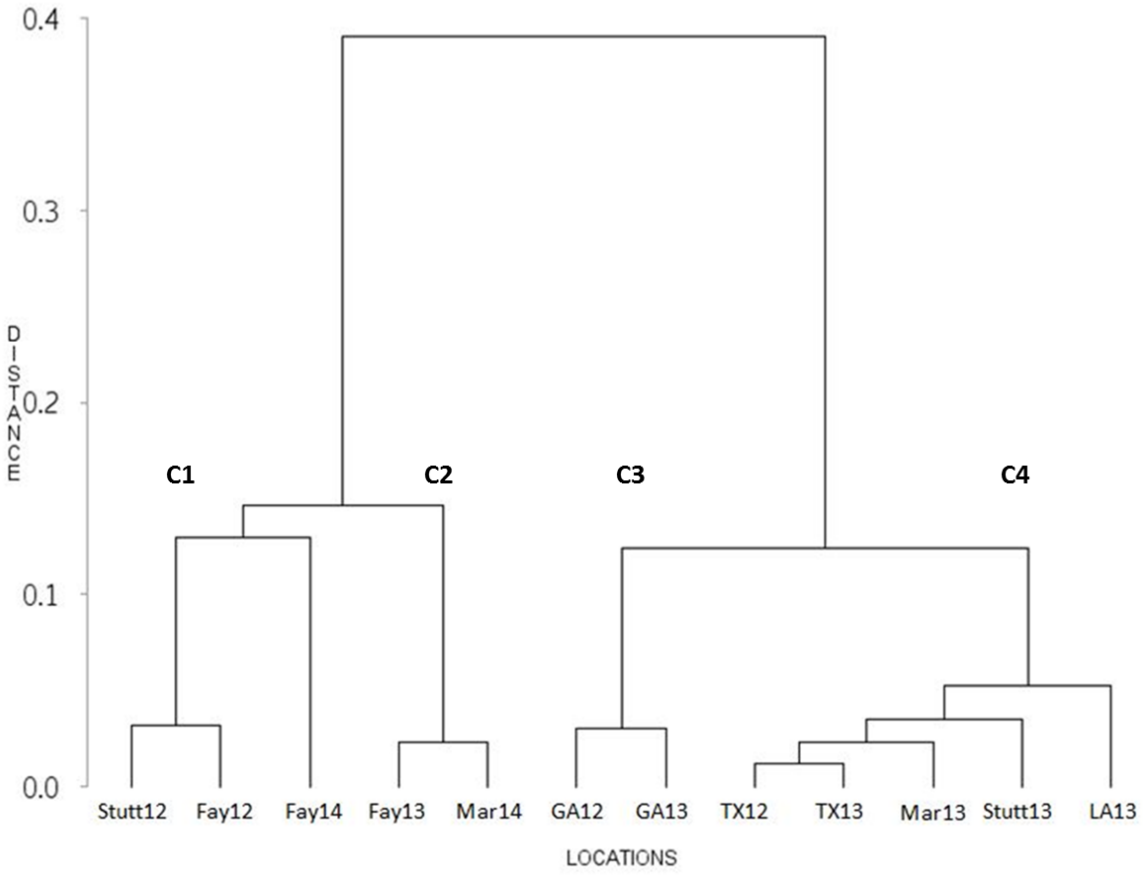


Figure1. Site regression analysis and environmental clustering for grain yield in the Pioneer 26R61 x AGS 2000 recombinant inbred lines across 12 site-years

Figure 2. QTL detected for the Pioneer 26R61 x AGS 2000 mapping population. QTL were reduced for resolution. Two LOD intervals are presented with favorable alleles from AGS 2000 presented in blue and favorable alleles from Pioneer 26R61 presented in red.

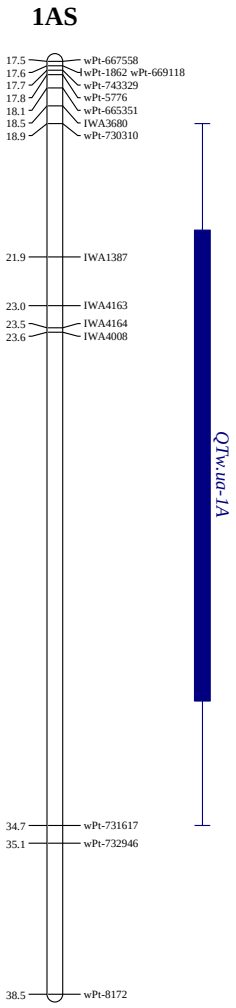


Figure 2. Continued

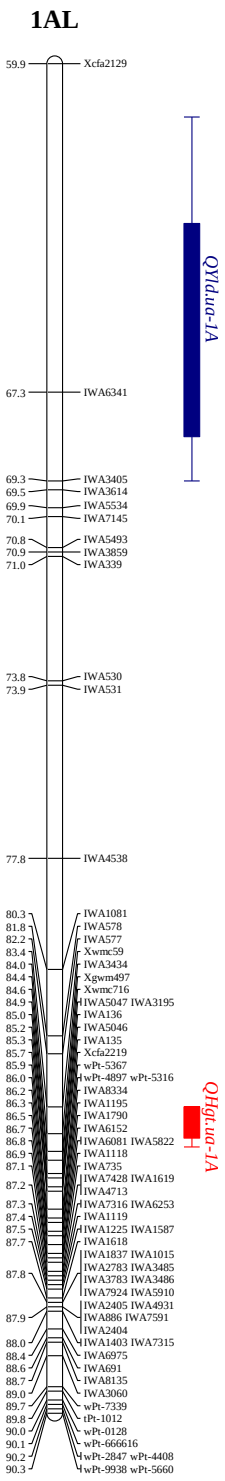


Figure 2. Continued

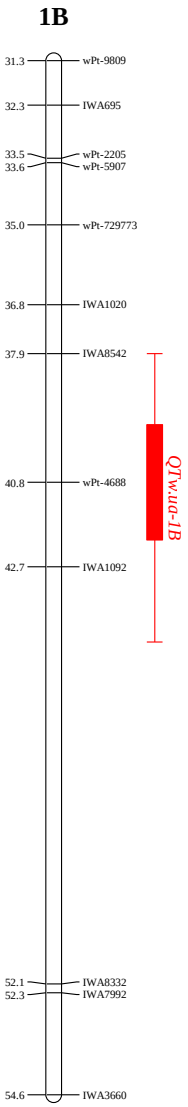
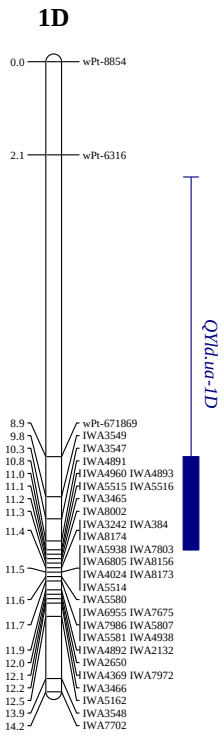


Figure 2. Continued



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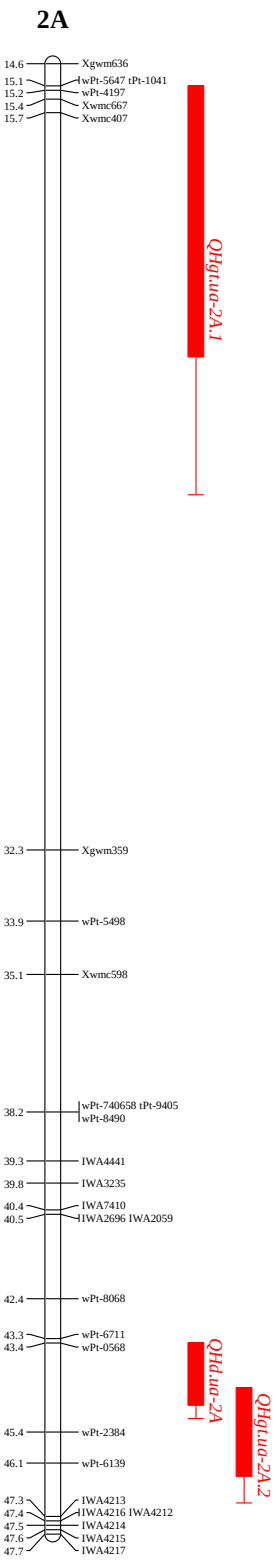

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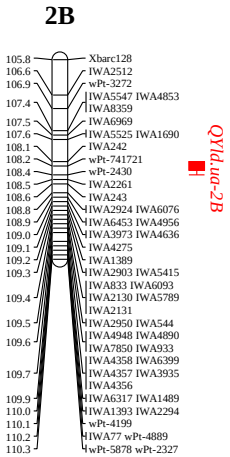

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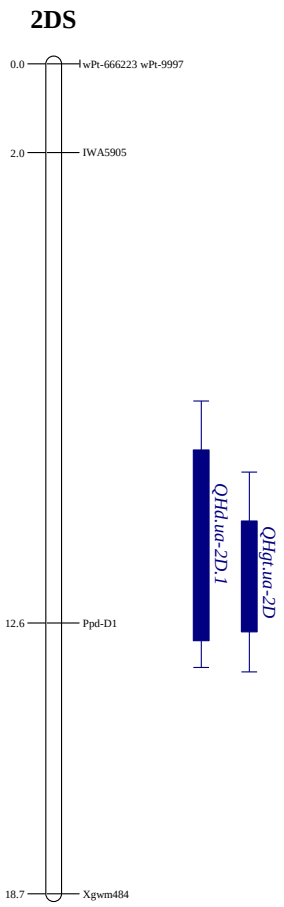

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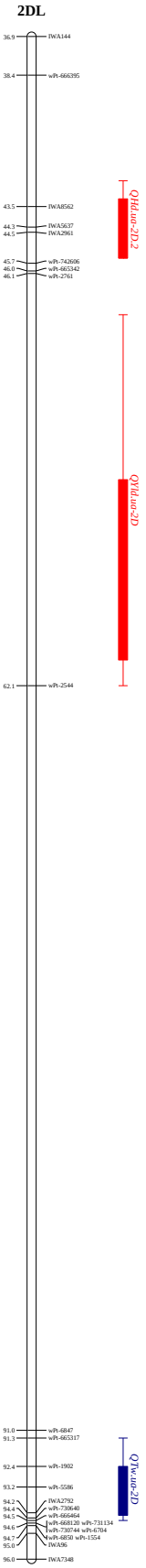
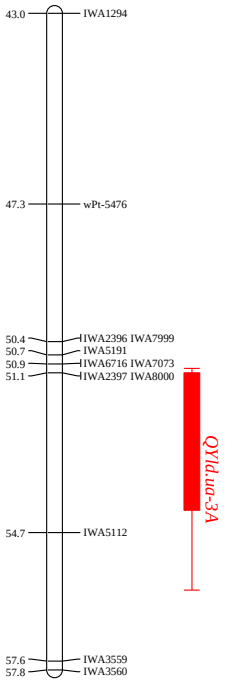


Figure 2. Continued **3A**



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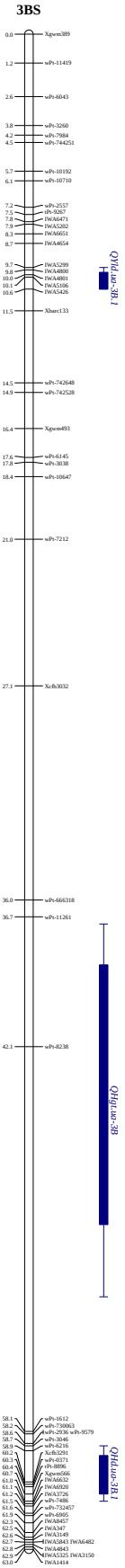

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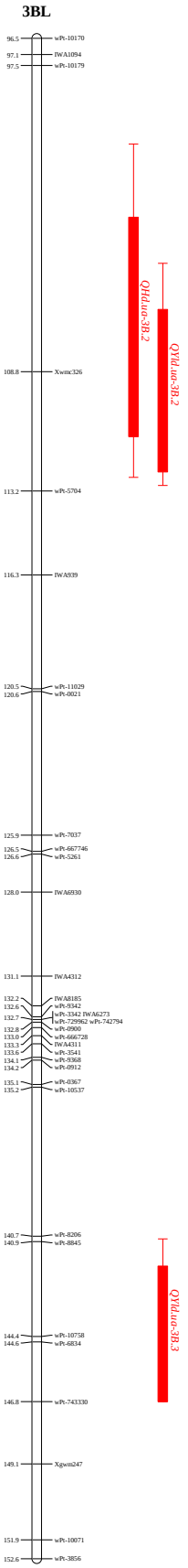
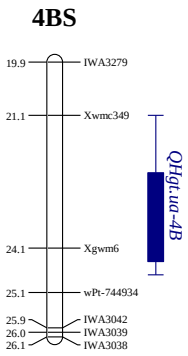


Figure 2. Continued



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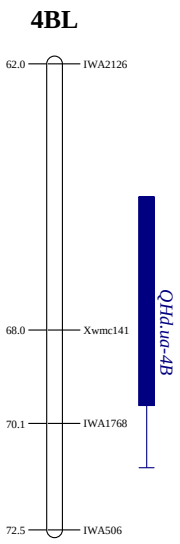


Figure 2. Continued

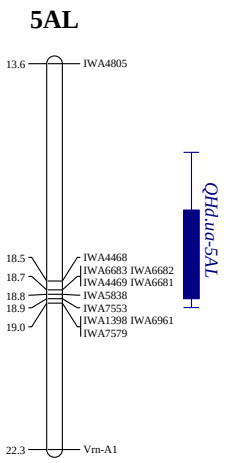


Figure 2. Continued

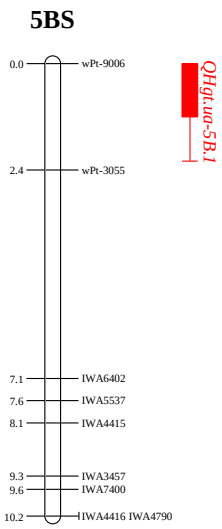
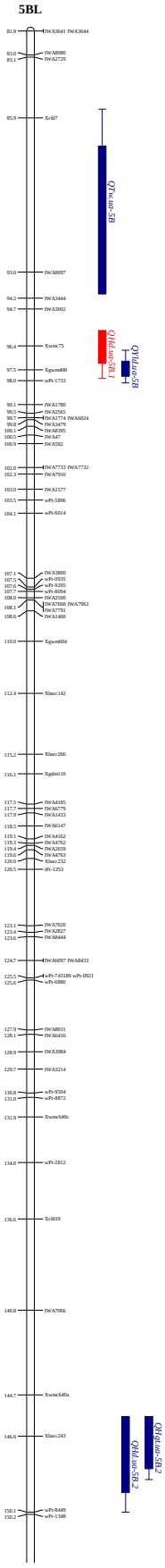


Figure 2. Continued



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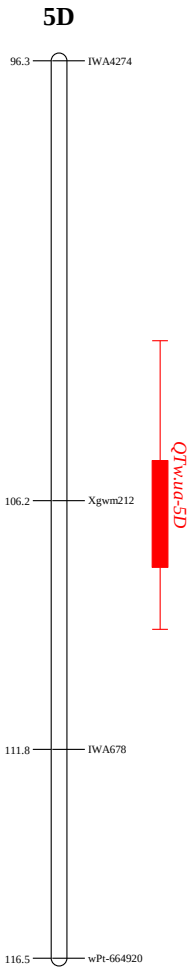
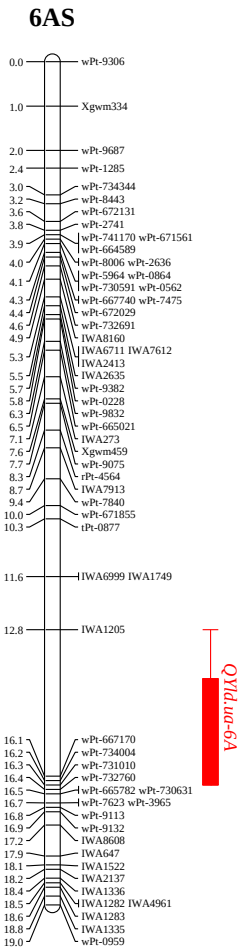


Figure 2. Continued



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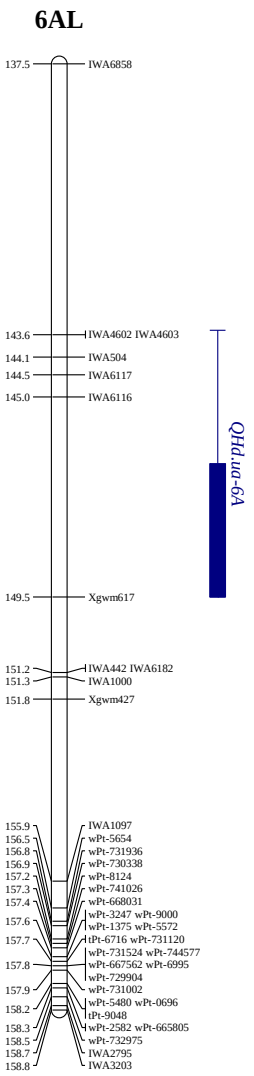

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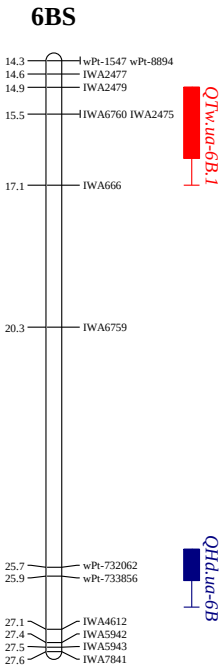

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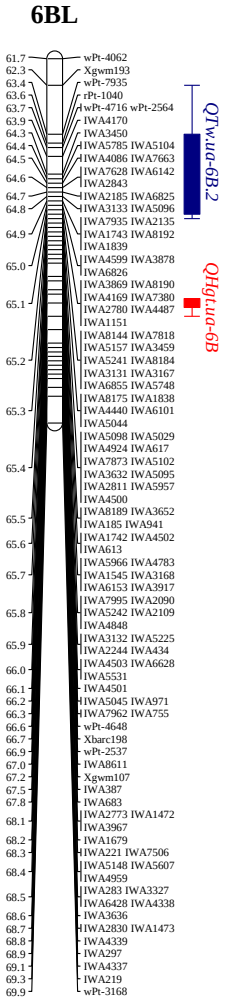

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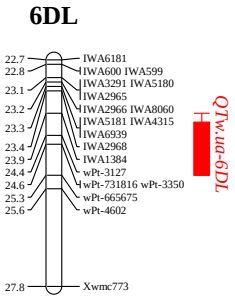

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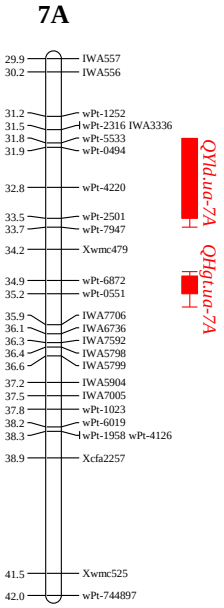

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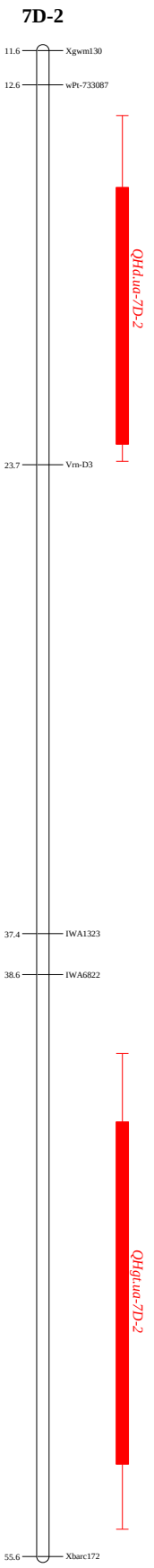

Chapter III

Genetic Mapping of NDVI Study

ABSTRACT

Spectral reflectance (SR) has been widely used as a tool to monitor crop growth and predict performance, particularly in abiotic-stressed environments that limit production. The goal of this study was to use normalized difference vegetative index (NDVI) as an indirect measurement of crop growth (biomass and canopy closure) in order to monitor changes over the growing season and identify quantitative trait loci (QTL) influencing these changes. To accomplish this, a wheat recombinant inbred line population consisting of 157 lines derived from a cross between 'Pioneer 26R61' and 'AGS2000' was used. These cultivars have shown to have high yield and broad adaptation in the southeastern region of the United States. The RIL population was grown over three years in three locations with a total of seven site-years. A randomized complete block design was used with two replications per location. Parental lines were replicated nine times in each block. The NDVI measurements varied per site-year with at least one measurement taken during both the vegetative and grain-filling stages at all locations. Vegetative biomass samples were harvested during early plant development. At maturity, 50 grain bearing tillers were harvested at ground level to determine yield components. Genetic variation and heritability of NDVI increased throughout the growing season as did correlations between NDVI and biomass or yield. Significant correlations ranged from r = -0.32 to 0.37 for NDVI development stages with yield, biomass at maturity and vegetative biomass. For individual environments, particularly those that had low production, correlations were as high as r = 0.72 for late season measurements of NDVI and yield. The QTL for NDVI were highly pleiotropic and were clustered in 14 genome regions across 11 of the 21 wheat chromosomes. Six of the 14 regions co-localized for both NDVI and biomass, with individual QTL explaining up to 14.7% of the phenotypic variation for NDVI.

INTRODUCTION

Wheat production areas, including the southeastern United States experience a variety of abiotic stresses, including high and low temperatures, drought and waterlogging that affect development throughout the growing season. These stresses affect wheat in different ways depending on timing, duration and intensity of the stress (Van Andel and Jager 1981). Heat stress is one of the most important constraints affecting up to 57% of the global wheat production (Kosina et al. 2007). Heat stress reduces grain yield up to 23% with as few as four days of exposure above optimal temperatures with maximum yield reduction up to 50% observed (Blum et al. 2001; Hawker and Jenner 1993; Randall and Moss 1990; Stone and Nicolas 1994). Drought stress is considered the largest constraint to wheat yield, affecting 42% of the global wheat production area (Kosina et al. 2007) with yield reductions ranging from 10 to 95% (Foulkes et al. 2007; Kilic and Yagbasanlar 2010; Ozturk and Aydin 2004; Sieling et al. 1994). Drought stress reduces wheat biomass by 40 to 60% due to decreased growth rates (Lopes and Reynolds 2011; Villegas et al. 2001). Waterlogging stress affects 12% of cultivated land in the United States. It is particularly important in Arkansas, with yield losses estimated up to 33% and up to 35% worldwide (Araki et al. 2012; Boyer 1982; Mason 2014; Setter and Waters 2003; Yaduvanshi et al. 2012).

Phenotyping in plant breeding programs is labor intensive and can be a limiting factor in the improvement of wheat grain yield and other adaptive traits. Normalized difference vegetative index (NDVI) and other spectral reflectance indices (SRIs) have the potential to serve as an aid to breeders for phenotypic selection across a range of environments (Busemeyer et al. 2013; Furbank 2009; Furbank and Tester 2011; Montes et al. 2007; Myles et al. 2009; White et al. 2012). These indices have the potential to allow breeders to evaluate a greater number of

genotypes in less time by providing a fast, non-subjective and non-destructive measurement of genetic potential. NDVI in particularly has been shown to be an effective way to measure early biomass accumulation, stay-green and chlorophyll loss (Bennett et al. 2012; Lopes and Reynolds 2012; Reynolds et al. 2007). Studies have shown the association of NDVI with biomass and grain yield, with moderate correlations reported in irrigated environments (Aparicio et al. 2000; Babar et al. 2006a; Erdle et al. 2013; Freeman et al. 2003; Gutierrez-Rodriguez et al. 2004; Prasad et al. 2007; Reynolds et al. 1999), drought stressed environments (Babar et al. 2006b; Gutierrez-Rodriguez et al. 2004; Reynolds et al. 2007) and heat stressed environments (Gutierrez et al. 2010; Hazratkulova et al. 2012; Lopes and Reynolds 2012; Reynolds et al. 2007).

Despite the utility of NDVI for monitoring crop growth, little is known about its genetic control. Pinto et al. (2010) reported QTL associated with NDVI measured at the vegetative stage on chromosomes 1B, 4A and 7B and at the grain-filling stage on chromosomes 2B, 4A and 7B. Bennett et al. (2012) also reported QTL for NDVI when measured at 25 days after emergence on chromosomes 1B, 2B, 3D, 4A, 5B and 7A. Despite these reports, information is still lacking on the genetic control of NDVI, particular at growth stages other than emergence and grain-filling, which influence growth and development and thus grain yield. Therefore, the aim of this study was to use NDVI as an indirect measurement of crop growth and to monitor changes in NDVI over the entire growing season across a range of environments. In addition, QTL associated with differences in NDVI will be identified which could aid breeders in selection of optimal plant growth for their target environments.

MATERIALS AND METHODS

Plant material

A recombinant inbred line (RIL) population consisting of 178 lines was used for this study. The RIL population was derived from a cross between two historically elite cultivars, 'Pioneer 26R61' and 'AGS2000', both of which are well-adapted to the southeastern United States. P26R61 (Omega 78/S76/Arthur 71/3/Stadler//Red-coat/Wisconsin 1/5/Coker 747/6/Pio. 2555 sib) was developed by Pioneer Hi-Bred and originally selected as a parental line because of its yield potential and resistance to stripe rust, leaf rust, soil borne wheat mosaic virus and Hessian fly. AGS2000 (Pio.2555/PF84301//FL 302; PI 612956) was developed by the University of Georgia and Florida Experiment Station (Hao et al. 2011). The cultivars have historically been used as yield checks in the Uniform Southern Soft Red Winter Wheat Nurseries (USSRWWN), and the development of the RIL population (referred to herein as P/A) has previously been described (Hao et al. 2011). The population segregates for genes related to photoperiod (*Ppd-D1, Ppd-B1 and Ppd-A1*) and vernalization (*vrn-A1 and vrn-B1*) and resistance to soil-borne mosaic virus (*Sbm1*). Following the 2011-2012 season, very early flowering and very late flowering lines were eliminated from further analyses to have a more narrow (~10 days) range of anthesis. The population does not segregate for any known genes controlling height (*Rht* genes) as both parents contain the *Rht-D1* dwarfing allele located on chromosome 4D.

Experimental Design

The P/A population was planted at two locations in the 2011-2012 growing season, three locations in the 2012-2013 growing season and three locations in the 2013-2014 growing season. Testing sites included Fayetteville, Stuttgart and Marianna, Arkansas. Mariana was not used as a testing site in 2011-2012 and Stuttgart was not harvested in 2013-2014 due to very poor stand and freeze damage. In 2011-2012 and 2012-2013 a randomized complete block design with two replications was used for all locations with both parental cultivars (P26R61 and AGS2000) and AGS2035 (2012-2013 only), a direct selection out of the P/A population, used as repeated checks every 20 plots. In 2013-2014, a Type-I augmented design with two replications was used which consisted of eight incomplete blocks with the two parents used as replicated checks.

All trials all years were drill seeded in seven row plots (1.5m width x 4.5m length) at a rate of 118 kg of seed hectare^{-1}. Planting and harvest dates and trial management varied based on recommendations at each location. Fayetteville received 150 kg of N ha^{-1} in either a single (2011-2012 and 2012-2013) or split application (2013-2014). Stuttgart received 170 kg of N ha^{-1} in a split application in all years. Mariana received 170 kg of N ha^{-1} plus 10 kg of ammonium sulfate ha^{-1} in a split application in all years. In 2011-2012 both Fayetteville and Stuttgart received a single fungicide application of 50 ml Tilt® (Syngenta Group Company) ha^{-1} to control for stripe rust.

Trait measurement

Grain yield (g m^{-2}) was determined at all locations by combine harvesting of whole plots and adjusting to 13% moisture. Prior to whole plot harvest, 50 spike-bearing culms were harvested per plot at maturity to estimate total biomass. Vegetative biomass was estimated from a 0.093m^2 sample taken at Feekes 5 in Fayetteville 2011-2012 and 2012-2013. Samples for biomass at maturity were not collected for Fayetteville 12-13 due to severe lodging. Normalized difference vegetative index (NDVI) measurements were taken with the Greenseeker® Handheld Sensor equipped with a Trimble® Nomad® 900 handheld computer for data-logging. All measurements were taken near mid-day and were repeated on multiple days throughout the growing season. While the number of measurements per site-year varied, at least one measurement was taken during both the vegetative and grain-filling stages at all locations. Readings were taken by walking between adjacent plots with the sensor over the center row at a height of 0.5m above the crop canopy, resulting in a plot coverage of 0.45m. For each plot, repeated measurements (20-25) were recorded and averaged to give one measurement per plot.

Statistical analyses

Statistical analyses were conducted using various procedures in SAS 9.3 (SAS Institute Inc. 2011, Cary, NC). Analysis of variance and significance testing were performed using PROC MIXED with genotype as a fixed effect. A separate analysis was conducted to calculate broad-sense heritability of measured traits from the variance components using TYPE3 sum of squares with all effects treated as random and using the following formula: $\sigma^2_G / (\sigma^2_G + \sigma^2_{GEI}/e + \sigma$

$^{2}_{E}/re)$, where σ^{2}_{G}, σ^{2}_{GEI}, σ^{2}_{E} are genotypic variance, genotype-by-environmental variance and error variance, respectively, and e and r are the numbers of environments and number of replications.

The NDVI measurements were initially assigned a Feekes growth stage and then categorized according to growing degree days (GDD). Growing degree days were calculated using the following formula starting from October 15th of the planting year; GDD = $(T_{max} + T_{min})/2 - T_{base}$, where T_{max} is maximum temperature, T_{min} is minimum temperature and T_{base} was 0°C. GDD were then used to assign ranges for seven development stages based on Miller et al. 2001, where establishment is 600-800 GDD, tillering is 900 – 1100 GDD, canopy closure is 1200 – 1400 GDD, anthesis is 1500 – 1700 GDD, seed fill is 1800 – 2100, dough stage is 2200 – 2400 GDD and maturity is 2600 - 3000. LSMeans were calculated for NDVI measurements across locations within each development stage and used for QTL analysis.

Genetic Map Construction

A genetic linkage map for the P26R61/AGS2000 population was previously developed by Hao et al. (2011). The original published linkage map contained 895 total loci spanning 2,659 centimorgans and included 747 diversity array technology (DArT) markers, 146 simple sequence repeats (SSR), and two powdery mildew resistance genes; Pm3-STS and Pm3a. Since the original published map, an additional 1,839 single nucleotide polymorphism (SNP) markers derived from the 9K Infinium SNP Array (Illumina Inc. San Diego, CA) have been integrated into the genetic map, bringing the total number of markers to 2,734 (Brown-Guedira, *In-prep*). This was carried out in collaboration with the USDA-ARS Regional Genotyping Centers in

Fargo, North Dakota and Raleigh, North Carolina. In total, there are 26 linkage groups which span all 21 wheat chromosomes.

QTL detection

With the exception of NDVI, best linear unbiased predictors (BLUPs) for line and trait combinations within individual environments and for each line and trait in a combined analysis using all environments were used for QTL analysis in WINQTL Cartographer version 2.5 (Wang et al. 2007). Due to missing data resulting from an unequal number of NDVI measurements per site-year and developmental stage, LSMEANS were used for QTL analysis for NDVI with measurements grouped and analyzed based on accumulated GDD. For QTL detection, single marker analysis was initially used to identify genetic markers significantly associated with phenotypic traits. Composite interval mapping (CIM) with forward/backward regression was then used to determine QTL positions at a significance threshold of $P < 0.05$ and a maximum of 25 control markers. Genomic regions where NDVI QTL were detected from two consecutive growth stages were deemed stable. As the focus of this study is genetic control of NDVI, QTL for yield (reported in Chapter 2) and biomass that did not co-localize with QTL for NDVI were not reported. Linkage groups were drawn using MapChart 2.2.

Results

Climatic and Phenotypic Data

Similar climatic conditions were observed in the same locations across years (Table 1). Stutt12 experienced the lowest amount of rainfall with 679.2 mm and 48 days with a maximum temperature above 30°C. Fay14 experienced the lowest average minimum temperature pre-anthesis and the lowest amount of days above 30°C throughout the growing season. These extreme environmental conditions resulted in Stutt12 and Fay14 being the two lowest yielding site-years (Table 2).

Mean grain yield of the RILs reached a maximum of 502 g m^{-2} in both Fay12 and Mar14. Minimum mean grain yield for the RILs of 339 g m^{-2} was observed in Stutt12. For biomass at maturity, the largest accumulation for the RILs occurred at Fay12 with 1266 g m^{-2} and Mar14 with 1522 g m^{-2}. Stutt12 had the lowest amount of biomass accumulation with 838 g m^{-2}. Overall, P26R61 accumulated more biomass than AGS2000 at all site-years with the exception of Fay14. For vegetative biomass, AGS2000 had a higher mean at both Fay12 and Fay13, the only site-years in which the trait was measured.

Analysis of variance showed environmental differences to explain the greatest amount of variation in the RILs for yield and biomass (Table 3), though variation for genotype, genotype by environment interaction and replication were also found to be significant with vegetative biomass the notable exception. Overall, broad-sense heritability estimates were low to moderate, ranging from $H^2 = 0.14$ for vegetative biomass to $H^2 = 0.44$ for yield.

NDVI and development

The NDVI measurements were taken throughout the growing season at seven wheat developmental stages determined based on growing degree days (GDD). When comparing subsequent growth stages, a significant change was always observed ($P < 0.05$) either in the positive or negative direction, with the exception of tillering to dough (Figure 1). A sharp increase in NDVI was observed between plant establishment and tillering followed by a smaller but significant increase from canopy closure to the onset of anthesis. A rapid decrease was observed after seed-fill as the plants began to reach physiological maturity. The RILs showed a similar trend throughout the growing season where significant differences were observed within each development stage as well across NDVI developments stages with the exception of NDVI at dough stage and NDVI at tillering where significant differences were not observed between the two (trend line in Figure 2, data not shown for significant differences). Environment explained the greatest amount of variation in the RILs for all development stages with the exception of NDVI at canopy closure where environmental significance was not detected (Table 3). Heritability and genetic variation for NDVI increased throughout the growing season, ranging from non-significant at establishment ($H^2 = 0.00$) to highly heritable near maturity ($H^2 = 0.68$).

Pearson's correlations were performed to examine the relationship of NDVI measurements at these seven developmental stages with yield and biomass. Positive correlations were observed between NDVI measurements taken at maturity and biomass at maturity ($r = 0.37$, $P < 0.0001$) and NDVI at tillering and vegetative biomass ($r = 0.28$, $P < 0.001$) (Table 4). The only association with yield was observed for NDVI at the dough stage ($r = 0.28$, $P < 0.001$). Correlations were much stronger in lower yielding, low biomass site-years, as shown for Fay14

(Table 5). Fay14 was late planted and resulted in low biomass accumulation, incomplete canopy closure and thus low yield potential resulting in the highest correlations of NDVI with yield and biomass. For yield, correlations ranged from 0.48 ($P < 0.0001$) for NDVI measurements taken during establishment to 0.72 ($P < 0.0001$) during grain-fill. Biomass at maturity was also significantly correlated with NDVI, ranging from $r=0.20$ to $r=0.54$ and increased throughout the growing season. Similar significant correlations were observed for Stutt12 and Stutt13, which were both low-yielding environments (data not shown).

NDVI QTL Summary

The NDVI QTL were detected using means within developmental stages across all environments in a combined analysis (Table 6, Figure 3). A total of 61 individual QTL were detected for NDVI which grouped into 14 clusters located on linkage groups 1A, 2BS, 2BL, 3A, 3B, 3D, 4DL, 5AS, 5D, 6AS, 6AL, 6BS, 6BL and 7B-1. All but four of the detected genome regions were significant for at least four NDVI measurements. The largest amount of variance explained by a single QTL was detected on chromosome 2B, with *QNant.ua-2BS* explaining 14.7% of the phenotypic variance for NDVI at anthesis and co-localizing with four other NDVI QTL. *QNem.ua-7B-1* explained the second largest amount of variance of all QTL detected at 11.3% with P26R61 contributing the favorable allele.

NDVI specific QTL

Eight of the 14 detected regions were specific for NDVI and did not show co-localization with QTL for either biomass or yield. Chromosome 4D showed the strongest influence on NDVI across developmental stages, being detected as significant for all seven NDVI measurements, with all favorable alleles contributed by AGS2000. *QNcan.ua-4DL* and *QNant.ua-4DL* accounted for the largest percentage of the phenotypic variance in this region, with R^2=10.0 and 10.7%, respectively. Six NDVI QTL co-localized on chromosome 3D with individual QTL explaining from 3.3% for *QNem.ua-3D* to 5.8% for *QNdou.ua-3D* of the phenotypic variance. On 6BS two later season NDVI measurements co-localized at marker *wPt-732062* marker, with AGS2000 contributing the favorable alleles for both *QNdou.ua-6BS* and *QNmat.ua-6BS*.

Co-localization of NDVI and Biomass QTL

Six regions showed co-localization for both NDVI and biomass QTL. On chromosome 5AS, QTL for six of the seven NDVI measurements co-localized with a QTL for biomass at maturity, *QMb.ua*-5A, which explained 2.2% of the phenotypic variance. Of the NDVI QTL in this region *QNem.ua-5AS* and *QNtil.ua-5AS* explained the most phenotypic variation with R^2 = 6.4 and 6.3%, respectively, and all favorable alleles contributed by AGS2000. P26R61 contributed all favorable alleles for six NDVI QTL on 6BL and for the biomass QTL *QMb.ua-6BL*, which co-localized at marker *IWA4717*. P26R61 also contributed all the favorable alleles for the seven QTL located in the second QTL cluster on chromosome 6AL. In this region, *QNem.ua-6AL* explained the largest amount of phenotypic variance at 6.5% while *QNant.ua-6AL* and *QNsf.ua-6AL* both accounted for 5.7%. A QTL for biomass at maturity, *QMb.ua-6AL* co-localized in this

region and explained 2.2% of the phenotypic variance. A second region on chromosome 6AS was significant for four NDVI QTL and co-localized with *QVb.ua-6AS* for vegetative biomass. Although AGS2000 contributed the favorable allele for all NDVI QTL in this region, P26R61 contributed the favorable allele for *QVb.ua-6AS* which explained 4.5% of the phenotypic variance of vegetative biomass. This was the only region detected where a negative pleiotropic relationship was observed. No QTL regions were identified which co-localized for both NDVI and yield.

DISCUSSION

Phenotypic analysis

P26R61 and AGS2000 are both considered to have wide adaptation to the growing region of the southeastern United States. AGS2000 was previously described by Hao et al. (2011) as the higher yielding parent and did so in four of the seven site-years reported here (Table 2). Though AGS2000 was higher yielding, P26R61 accumulated more biomass throughout the growing season with the exception of Fayetteville 13-14. These results are confirmed by measurements of NDVI, where despite similar measurements of NDVI for AGS2000 and P2R61 early in the season, end of season measurements were greater in P26R61 (Figure 1). Few studies have examined changes in NDVI over the entire developmental process of a wheat crop and how changes correlate with yield and biomass traits. In this study, low to moderate correlations were observed when looking at NDVI measurements across all site-years, with the highest correlations observed between NDVI measurements taken near maturity ($r = 0.37$, $P < 0.0001$), closer to

when biomass sampling was done (Table 4). A low but significant correlation was also observed between NDVI measurements at dough stage and grain yield ($r = 0.28$, $P < 0.001$) in the combined analysis. These correlations were much stronger in a lower yielding, lower biomass environment (Table 5) with correlations as high as $r = 0.72$ between late season NDVI measurements and grain yield. This is in agreement with previous research by Gutierrez-Rodriguez et al. (2004) who reported moderate correlations between NDVI and yield ($r = 0.54$) under similar conditions. Similarly, using NDVI as a measurement of stay-green, Lopes and Reynolds (2012) reported moderate correlations between NDVI taken at maturity and yield with ($r = 0.40$). Gutierrez et al. (2010) also reported similar results for NDVI and yield when plant development is affected by abiotic stress with correlations as high as $r = 0.82$.

QTL analysis

In contrast to previous studies, the objective here was to identify genome regions associated with NDVI measurements taken over the entire growing season, as oppose to previous reports that focus on either emergence or grain-filling as a "snap-shot" of development (Bennett et al. 2012; Pinto et al. 2010). A total of 61 individual QTL were detected for NDVI which grouped into 14 clusters. Most genome regions were found to be associated with at least four different developmental stage measurements of NDVI, indicating a strong effect of genes within these QTL regions. The QTL explaining the largest amount of variance for NDVI was detected on chromosome 2B, explaining 14.7% (*QNant.ua-2BS*) of the phenotypic variance and co-localizing with four additional NDVI QTL and a QTL for vegetative biomass (*QVb.ua-2BS*). Bennett et al. (2012) reported a QTL for early season NDVI (*Q.Ndvi.aww-2B*) distal to this region but in close proximity of markers *wPt-7360, wPt-2135* and *wPt-3378* that were consistent across both genetic

maps. Similarly, a QTL cluster on chromosome 7B-1, which contained a significant QTL for NDVI at establishment (*QNem.ua-7B-1*, R^2 = 11.3%) as well as other QTL for NDVI at tillering, anthesis and seed fill was in agreement with Pinto et al. (2010) who reported a QTL for NDVI during grain-filling on chromosome 7B that explained 5.6% of the phenotypic variance. Therefore, it appears that the NDVI QTL with the largest effects (*QNem.ua-7B-1*, R^2 = 11.3% and *QNant.ua-2BS*, R^2 = 14.7%) may be detectable in different genetic backgrounds.

With the exception of these two examples, little can be confirmed regarding the QTL identified in this study as previous research is lacking in this area. Future work is needed to identify candidate genes within these regions and what impact selection for these QTL would have on improving yield in a breeding program. No genomic regions were identified which co-localized for both NDVI and yield in the combined analysis, which is not surprising given the low phenotypic correlation between the two traits. Sporadic co-localization was observed when looking at data for individual environments (data not shown), particularly in low-yielding environments, but the effects of these QTL were not robust enough to be detected across environments. Regardless, the pleiotropic nature of these regions, where potentially a single gene within a QTL region may affect growth and development throughout the season, make them interesting for future genetic improvement and they may serve as potential targets for marker assisted selection to target growth to meet the demands of a target environment.

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Table 1. Summary of climatic data across seven site-years

Site Year	Average Min Temp (°C)		Average Max Temp (°C)		Rainfall (mm)		Days > 30°C	
	Pre Anthesis	Post Anthesis	Pre Anthesis	Post Anthesis	Pre Anthesis	Post Anthesis	Pre Anthesis	Post Anthesis
Fayetteville 11-12	2.8	14	15.3	27.6	555.8	136.9	0	34
Fayetteville 12-13	0.4	11.8	13.8	24.3	409.7	370.3	0	19
Fayetteville 13-14	-1.8	12.3	11.8	24.4	508.5	268.0	0	12
Marianna 12-13	4.1	14.7	14.2	25.7	630.2	351.8	0	31
Marianna 13-14	2.3	15.4	12.9	26.0	467.6	437.4	3	35
Stuttgart 11-12	6.9	17.5	17.2	28.7	529.8	149.4	4	44
Stuttgart 12-13	4.7	15.7	14.5	26.1	497.8	283.5	0	32

[a]Min indicated Minimum, Max indicated Maximum

Table 2. Summary for yield and biomass traits measured in the Pioneer 26R61 x AGS 2000 recombinant inbred lines across seven site-years

	Yield (g m^{-2})				Biomass at Maturity (g m^{-2})				Vegetative Biomass (g m^{-2})			
Site Year	AGS 2000	Pioneer 26R61	RIL	RIL Range	AGS 2000	Pioneer 26R61	RIL	RIL Range	AGS 2000	Pioneer 26R61	RIL	RIL Range
Fayetteville 11-12	543	520	502	280 - 659	1243	1334	1266	791 - 1781	3.61	3.05	3.54	0.62 - 10.6
Fayetteville 12-13	456	471	435	101 - 705	-	-	-	-	21.20	20.36	22.56	13.81 - 39.29
Fayetteville 13-14	419	394	386	190 - 545	1270	1168	1214	690 - 1686	-	-	-	-
Marianna 12-13	410	433	391	181 - 567	1178	1285	1201	555 - 2853	-	-	-	-
Marianna 13-14	513	529	502	290 - 613	1383	1463	1522	827 - 2057	-	-	-	-
Stuttgart 11-12	373	336	339	165 - 477	811	838	803	404 - 1280	-	-	-	-
Stuttgart 12-13	421	399	397	193 - 557	1099	1166	1126	524 - 3921	-	-	-	-

Table 3. Percent of total variance due to genotype, environment, genotype x environment (G X E), replication and error for the Pioneer 26R61 x AGS 2000 recombinant inbred lines.

Trait	No. of Environments	Genotype (G)	Environment (E)	G X E	Rep(Loc)	Residual	H^2
NDVI at Establishment	3	0.00	0.74****	0	0.03****	0.24	0.00
NDVI at Tillering	4	0.01	0.92****	0.01****	0	0.06	0.07
NDVI at Canopy Closure	4	0.00	0.86	0.03****	0.01****	0.10	0.14
NDVI at Anthesis	5	0.01****	0.70****	0.01	0.02****	0.27	0.27
NDVI at Seedfill	4	0.00	0.72****	0.07****	0	0.21	0.03
NDVI at Dough Stage	4	0.02****	0.38****	0	0.01***	0.59	0.20
NDVI at Maturity	3	0.13****	0.63****	0.13****	0.02****	0.09	0.68
Yield (g/m2)	7	0.04****	0.40****	0.12****	0.02****	0.43	0.44
Maturity Biomass	6	0.02****	0.53****	0.04***	0.02****	0.38	0.30
Vegetative Biomass	2	0	0.95****	0	0	0.04	0.14

* Significant at P = 0.05
** Significant at P = 0.01
*** Significant at P = 0.001
**** Significant at P = 0.0001

Table 4. Correlations of NDVI taken in seven development stages with yield and biomass traits in the Pioneer 26R61 x AGS 2000 recombinant inbred lines

Development	Growing Degree Days	Yield	Maturity Biomass	Vegetative Biomass
Establishment	600 - 800	0.06	-0.05	0.08
Tillering	900 - 1100	0.04	0.02	0.26***
Canopy Closure	1200 - 1400	0.09	0.06	0.18*
Anthesis	1500 - 1700	0.07	0.30****	-0.17*
Seed Fill	1800 - 2100	0.05	0.19**	-0.10
Dough Stage	2200 - 2400	0.28***	0.13	-0.21**
Maturity	2600 - 3000	-0.01	0.37****	-0.32****

*Significant at P = 0.05
** Significant at P = 0.01
*** Significant at P = 0.001
****Significant at P = 0.0001

Table 5. Correlation of yield and biomass at maturity with NDVI taken at growing degree days (GDD) in the Pioneer 26R61 x AGS 2000 recombinant inbred lines Fayetteville 13-14

GDD	Yield	Biomass at Maturity
700	0.48***	0.20*
800	0.61***	0.35***
1000	0.63***	0.40***
1300	0.69***	0.49***
1500	0.69***	0.48***
1700	0.72***	0.54***

*Significant at P = 0.01
***Significant at P = 0.0001

Table 6. Summary of QTL detected for the P26R61 x AGS 2000 recombinant inbred lines

Chromosome QTL name	Trait	Linked Marker	Position (cM)	LOD	R^2	Additive Effect	Source[a]
Chromosome 1A							
QNsf.ua-1A	NDVI at seedfill	wPt-8172	37.1	3.1	0.033	0.048	A
QNem.ua-1A	NDVI at establishment	wPt-8172	38.5	3.6	0.030	0.020	A
QNtil.ua-1A	NDVI at tillering	wPt-8172	38.5	3.9	0.043	0.042	A
Chromosome 2BS							
QNtil.ua-2BS	NDVI at tillering	wPt-0473	120.7	7.1	0.067	0.052	A
QNant.ua-2BS	NDVI at anthesis	wPt-0694	121.0	14.9	0.147	0.094	A
QNem.ua-2BS	NDVI at establishment	Xbarc332	123.6	10.6	0.088	0.033	A
QNcan.ua-2BS	NDVI at canopy closure	Xbarc332	123.6	2.9	0.025	0.042	A
QNsf.ua-2BS	NDVI at seedfill	Xbarc332	123.6	7.1	0.068	0.069	A
QVb.ua-2BS	Vegetative biomass	Xbarc332	123.6	5.4	0.045	0.034	A
Chromosome 2BL							
QNmat.ua-2BL	NDVI at maturity	IWA2046	170.9	3.8	0.036	0.019	A
QNdou.ua-2BL	NDVI at dough stage	wPt-8916	172.7	6.0	0.056	0.049	A
Chromosome 3A							
QNem.ua-3A	NDVI at establishment	IWA4257	3.0	6.7	0.059	0.027	A
QNant.ua-3A	NDVI at anthesis	Xwmc532	4.0	2.8	0.028	0.041	A
QNcan.ua-3A	NDVI at canopy closure	wPt-1939	6.5	3.3	0.028	0.037	A
QNsf.ua-3A	NDVI at seedfill	wPt-1939	6.5	3.6	0.033	0.048	A
Chromosome 3B							
QNsf.ua-3B	NDVI at seedfill	wPt-730063	58.2	2.6	0.024	0.040	P
QNdou.ua-3B	NDVI at dough stage	wPt-730063	58.2	4.6	0.042	0.041	P
QNmat.ua-3B	NDVI at maturity	wPt-730063	58.2	7.4	0.065	0.027	P
QNant.ua-3B	NDVI at anthesis	IWA6920	61.1	3.2	0.027	0.040	P
Chromosome 3D							
QNcan.ua-3D	NDVI at canopy closure	Xgdm8	0.9	4.6	0.041	0.043	P
QNant.ua-3D	NDVI at anthesis	Xgdm8	0.9	6.0	0.052	0.056	P
QNdou.ua-3D	NDVI at dough stage	Xgdm8	0.9	6.2	0.058	0.048	P
QNem.ua-3D	NDVI at establishment	IWA5695	1.9	4.4	0.033	0.020	P
QNtil.ua-3D	NDVI at tillering	IWA5695	1.9	4.3	0.049	0.043	P
QNsf.ua-3D	NDVI at seedfill	IWA5695	1.9	3.7	0.034	0.047	P
Chromosome 4DL							
QNem.ua-4DL	NDVI at establishment	IWA1783	6.0	5.5	0.044	0.023	A
QNtil.ua-4DL	NDVI at tillering	IWA1783	6.0	4.6	0.054	0.047	A
QNcan.ua-4DL	NDVI at canopy closure	IWA1783	6.0	10.0	0.100	0.068	A
QNant.ua-4DL	NDVI at anthesis	IWA1783	6.0	11.0	0.107	0.082	A
QNsf.ua-4DL	NDVI at seedfill	IWA1783	6.0	4.8	0.047	0.056	A
QNdou.ua-4DL	NDVI at dough stage	IWA1783	6.0	5.4	0.051	0.044	A
QNmat.ua-4DL	NDVI at maturity	IWA1783, Xgwm609	9.8	7.7	0.077	0.027	A
Chromosome 5AS							

QNcan.ua-5AS	NDVI at canopy closure	wPt-4131	31.0	8.9	0.083	0.064	A
QNant.ua-5AS	NDVI at anthesis	wPt-4131	31.0	6.3	0.056	0.061	A
QNem.ua-5AS	NDVI at establishment	wPt-4131	31.2	7.9	0.064	0.029	A
QNtil.ua-5AS	NDVI at tillering	wPt-4131	31.2	5.6	0.063	0.052	A
QNsf.ua-5AS	NDVI at seedfill	wPt-4131	31.2	3.2	0.030	0.047	A
QNdou.ua-5AS	NDVI at dough stage	wPt-4131	31.2	3.1	0.032	0.040	A
QMb.ua-5AS	Maturity biomass	wPt-4131	31.2	2.5	0.022	3.580	A
Chromosome 5D							
QNem.ua-5D	NDVI at establishment	Xwmc443	168.9	6.7	0.063	0.029	P
QNcan.ua-5D	NDVI at canopy closure	Xwmc443	170.3	4.3	0.037	0.043	P
QNant.ua-5D	NDVI at anthesis	rPt-3825	172.7	2.5	0.021	0.035	P
Chromosome 6AS							
QNem.ua-6AS	NDVI at establishment	wPt-672131	3.6	4.1	0.039	0.023	A
QNsf.ua-6AS	NDVI at seedfill	wPt-672131	3.6	3.1	0.028	0.045	A
QNant.ua-6AS	NDVI at anthesis	wPt-0228	5.8	2.9	0.028	0.042	A
QVb.ua-6AS	Vegetative biomass	wPt-9075	7.7	4.5	0.045	0.035	P
QNdou.ua-6AS	NDVI at dough stage	wPt-671855	10.0	3.6	0.039	0.039	A
Chromosomes 6AL							
QMb.ua-6AL	Maturity biomass	IWA259, IWA260	105.9	2.6	0.022	3.344	P
QNcan.ua-6AL	NDVI at canopy closure	Xwmc179	107.7	4.2	0.037	0.043	P
QNem.ua-6AL	NDVI at establishment	wPt-5310	118.7	7.9	0.065	0.031	P
QNtil.ua-6AL	NDVI at tillering	wPt-5310	118.7	2.8	0.031	0.036	P
QNant.ua-6AL	NDVI at anthesis	wPt-5310	118.7	6.3	0.057	0.060	P
QNsf.ua-6AL	NDVI at seedfill	wPt-5310	118.7	6.0	0.057	0.066	P
Chromosome 6BS							
QNdou.ua-6BS	NDVI at dough stage	wPt-732062	25.7	7.8	0.072	0.056	A
QNmat.ua-6BS	NDVI at maturity	wPt-732062	25.7	3.1	0.025	0.018	A
QVb.ua-6BS	Vegetative biomass	wPt-663764	35.1	3.9	0.032	0.031	A
Chromosome 6BL							
QNsf.ua-6BL	NDVI at seedfill	IWA4717	103.9	2.6	0.032	0.049	P
QNem.ua-6BL	NDVI at establishment	IWA4717	105.9	3.7	0.031	0.020	P
QNtil.ua-6BL	NDVI at tillering	IWA4717	105.9	3.1	0.036	0.039	P
QNcan.ua-6BL	NDVI at canopy closure	IWA4717	105.9	5.2	0.050	0.048	P
QNant.ua-6BL	NDVI at anthesis	IWA4717	105.9	8.9	0.089	0.074	P
QNmat.ua-6BL	NDVI at maturity	IWA4717	105.9	3.7	0.034	0.019	P
QMb.ua-6BL	Maturity biomass	IWA4717	108.7	3.3	0.033	4.349	P
Chromosome 7B-1							
QNem.ua-7B-1	NDVI at estabishment	IWA3506	19.2	12.8	0.113	0.039	P
QNant.ua-7B-1	NDVI at anthesis	IWA3506	19.2	4.9	0.042	0.051	P
QNsf.ua-7B-1	NDVI at seedfill	IWA3506	19.2	5.4	0.050	0.062	P
QNtil.ua-7B-1	NDVI at tillering	IWA1315	21.2	6.3	0.073	0.052	P

[a]Favorable allele designated by a P for Pioneer 26R61 and an A for AGS 2000

Figure 1. Normalized difference vegetative index (NDVI) changes for the mean of Pioneer 26R61 x AGS 2000 recombinant inbred lines and parental lines throughout the growing season

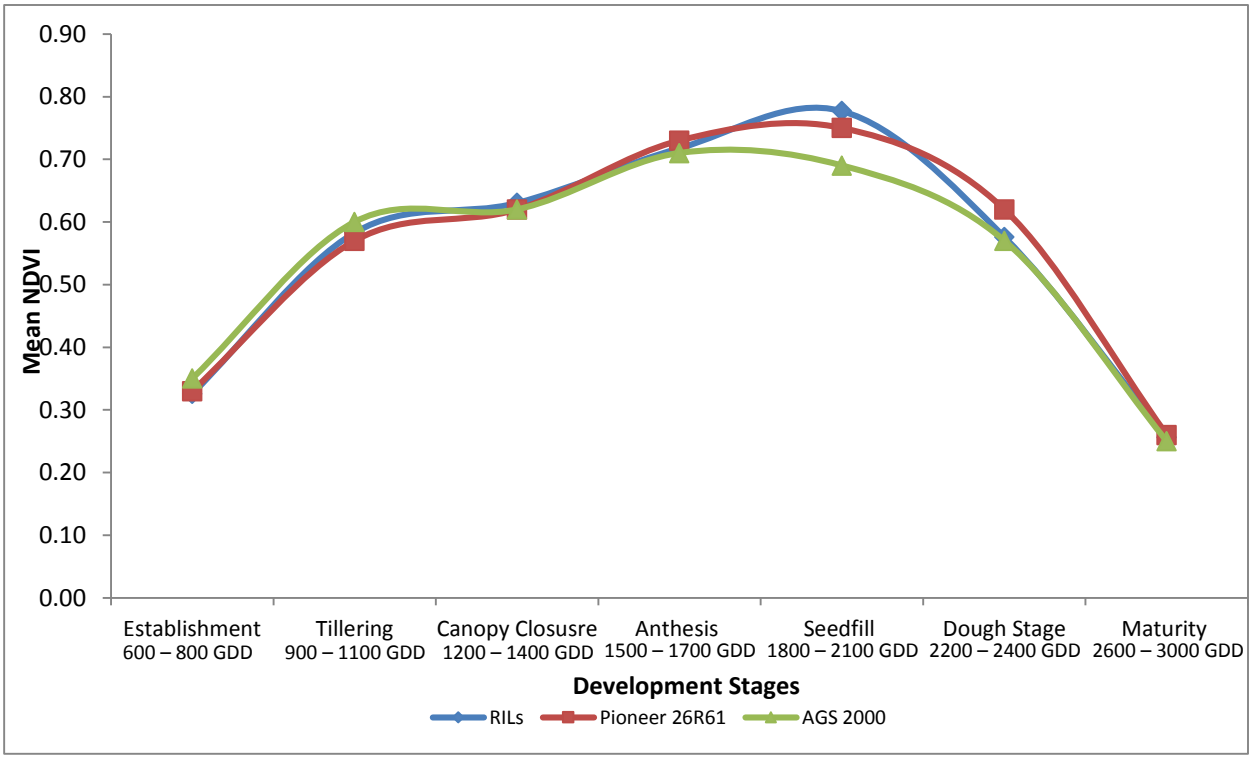


Figure 2. Normalized difference vegetative index (NDVI) changes and range for individual Pioneer 26R61 x AGS 2000 recombinant inbred lines throughout the growing season

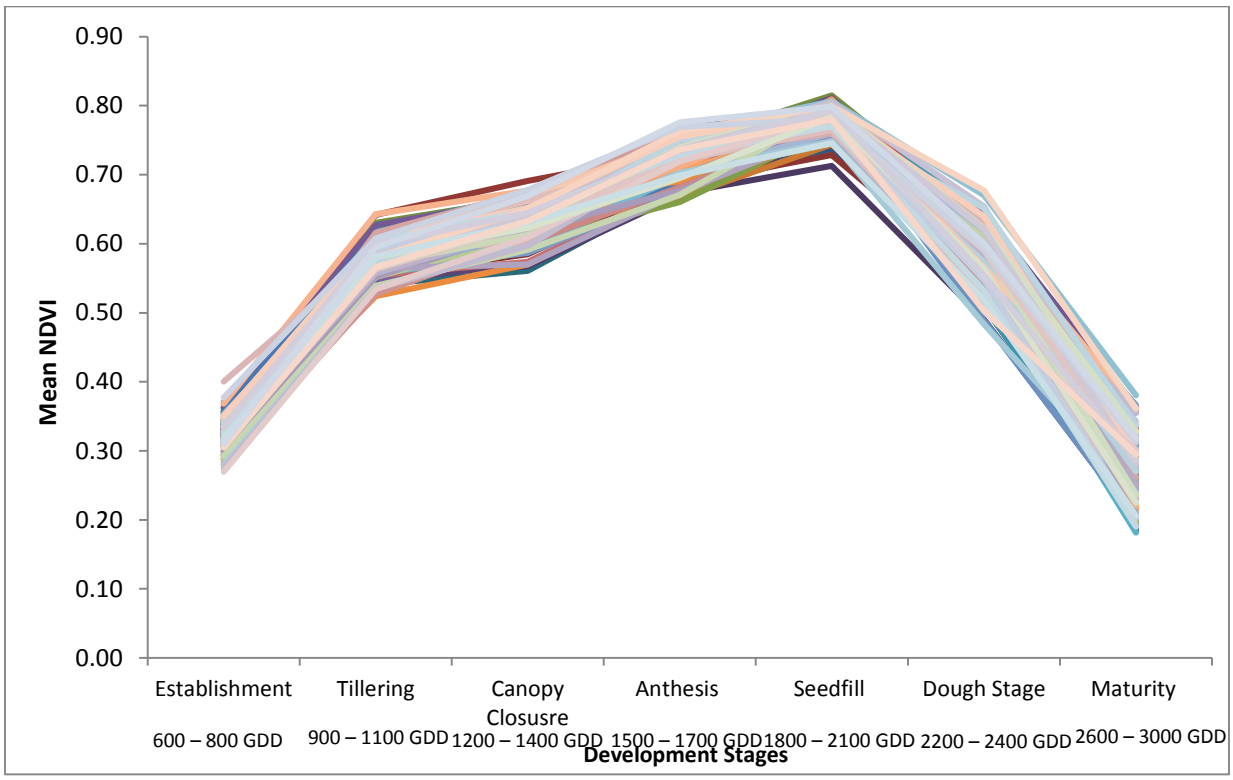


Figure 3. QTL detected for the Pioneer 26R61 x AGS 2000 mapping population. QTL were reduced for resolution. Two LOD intervals are presented with favorable alleles from AGS 2000 presented in blue and favorable alleles from Pioneer 26R61 presented in red. Reference Table 6 for details of detected QTL.

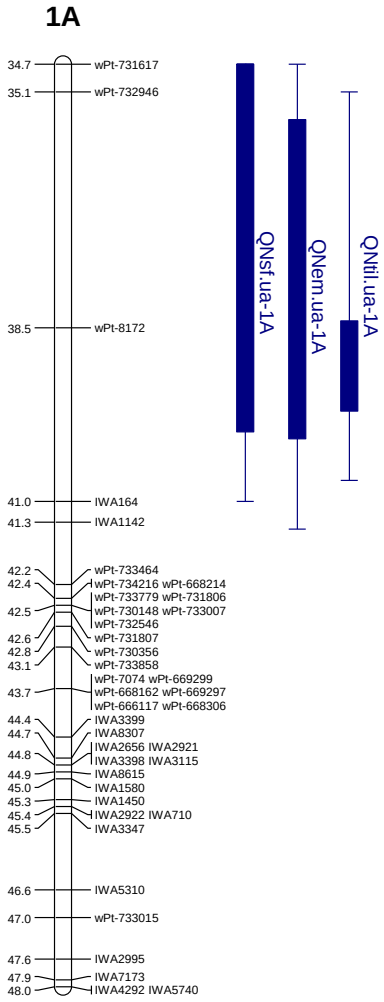


Figure 3. Continued

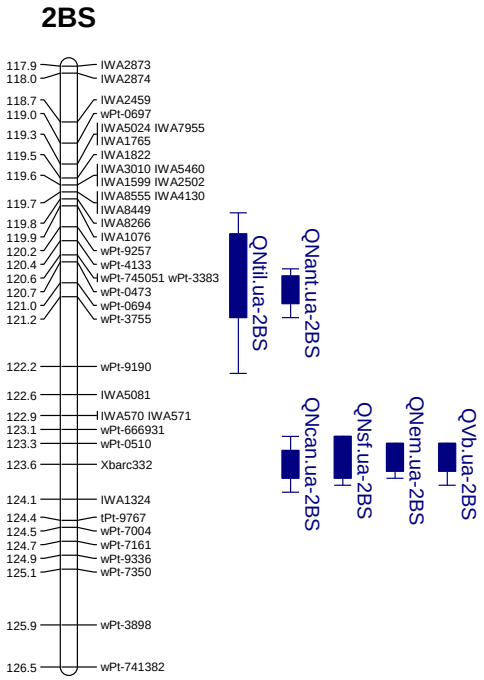


Figure 3. Continued

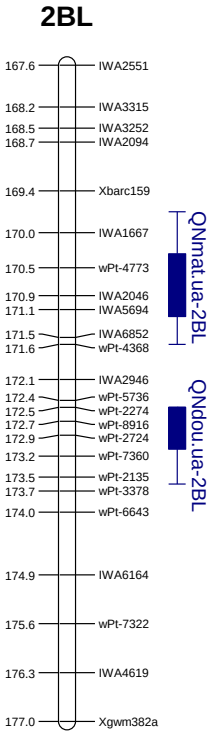


Figure 3. Continued

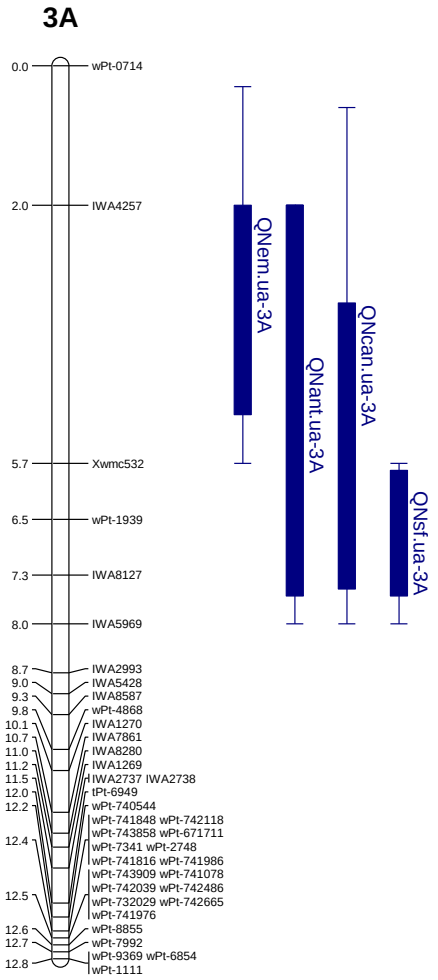


Figure 3. Continued

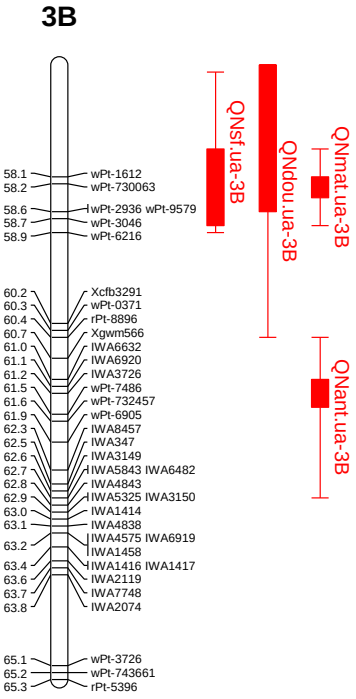


Figure 3. Continued

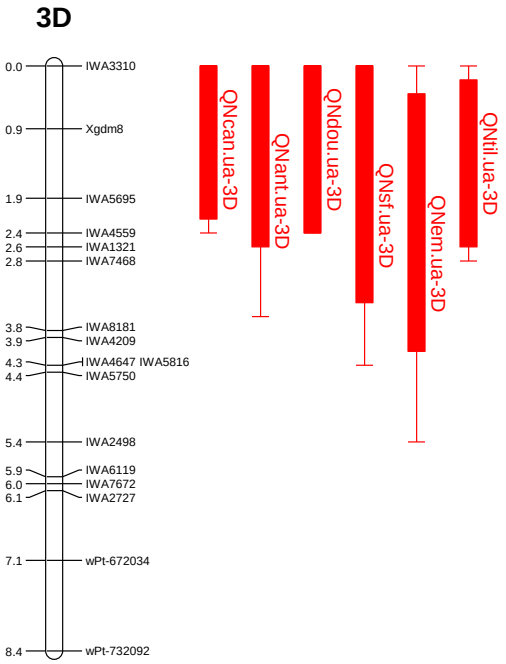


Figure 3. Continued

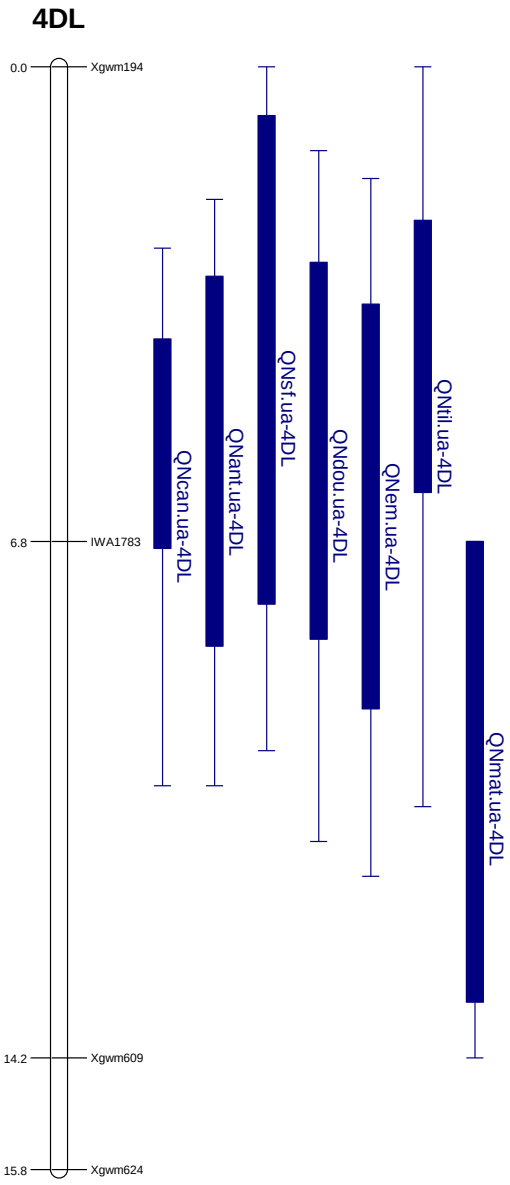


Figure 3. Continued

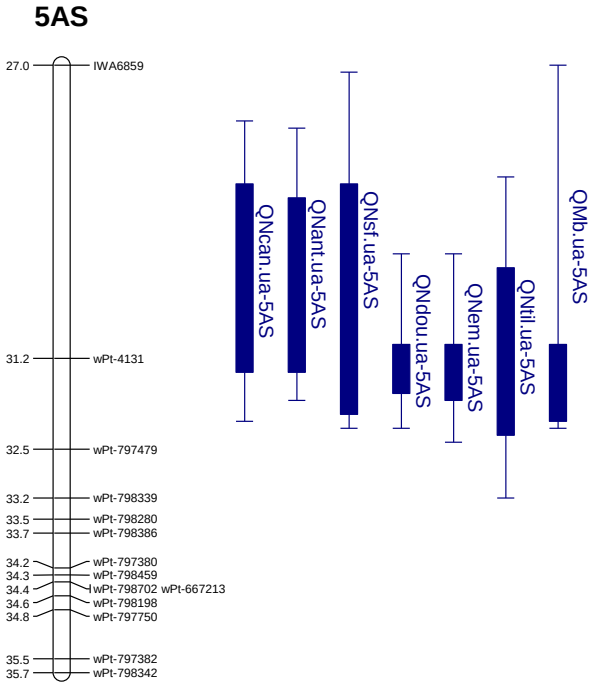


Figure 3. Continued

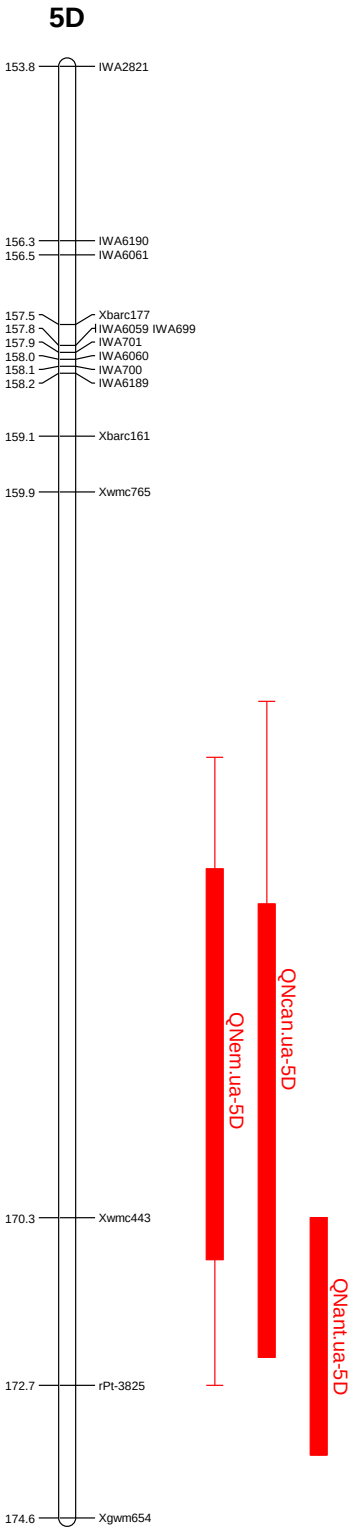


Figure 3. Continued

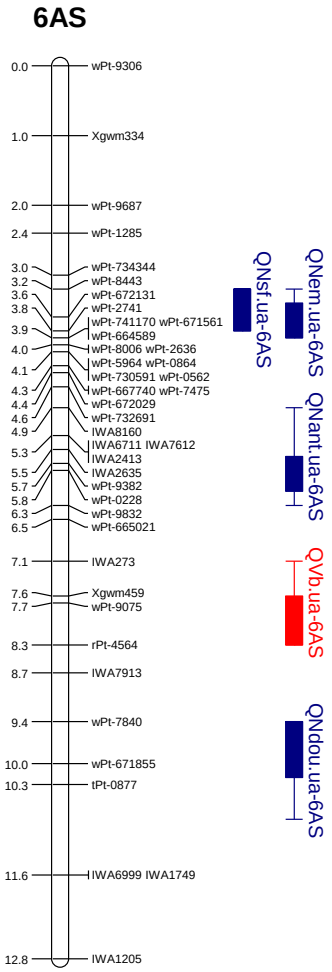


Figure 3. Continued

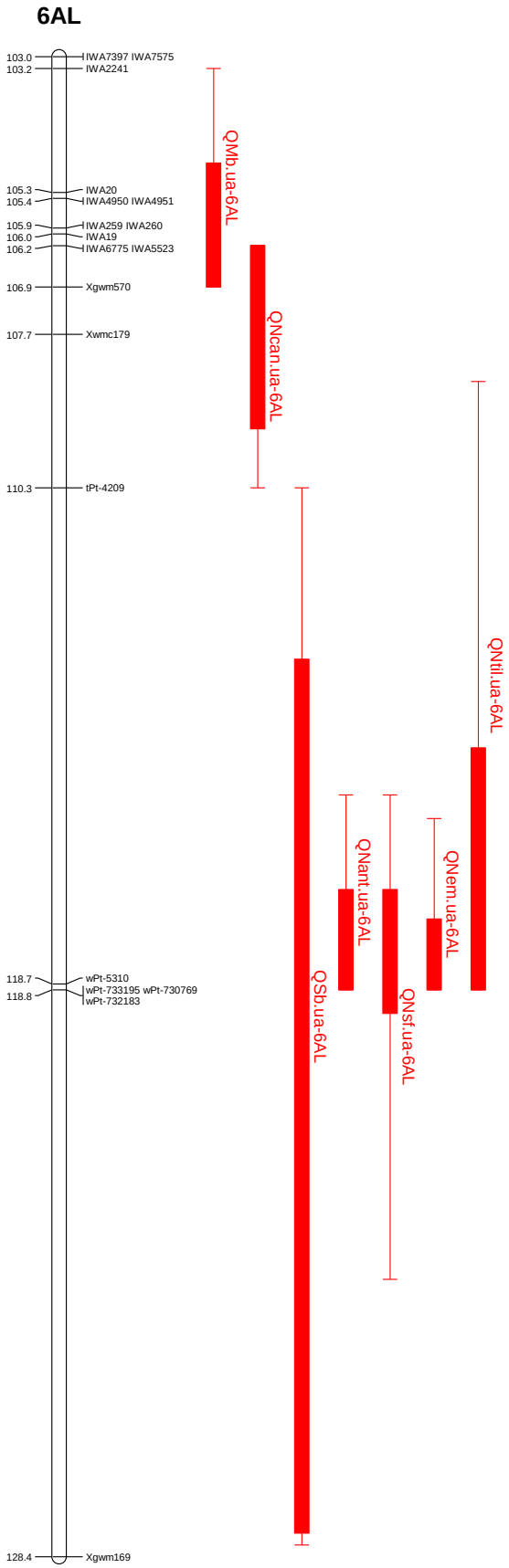


Figure 3. Continued

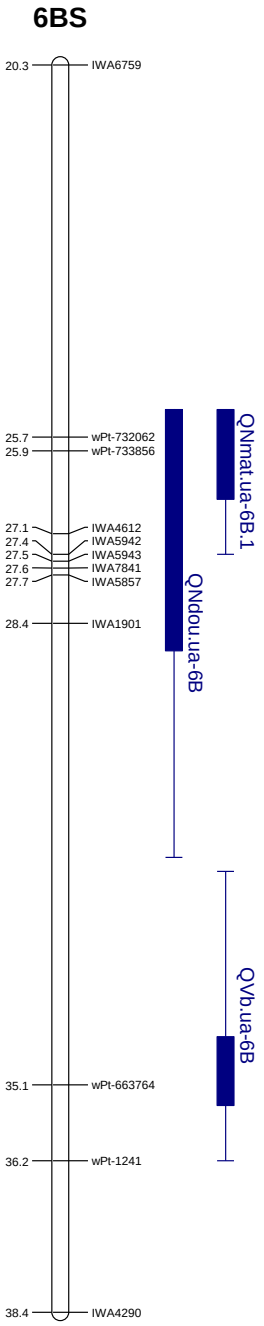


Figure 3. Continued

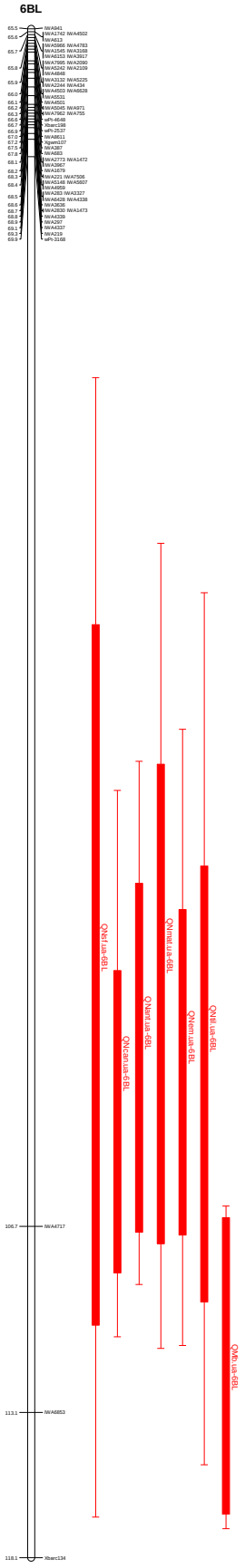
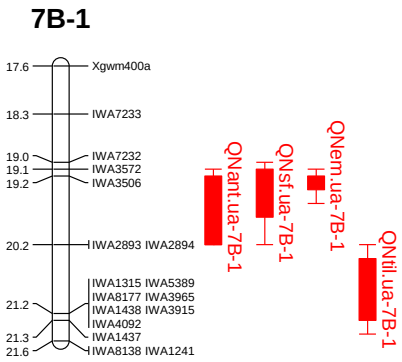


Figure 3. Continued



Chapter IIII

Overall Conclusion

Overall Conclusions

The lack of knowledge on the genetic control of yield and related traits for soft red winter wheat is a constraint to breeders in the southeastern U.S. This study was aimed at narrowing the knowledge gap for breeders in this region by analyzing a soft red winter wheat mapping population across a range of environments.

For the genetic mapping of yield and test weight study, QTL number ranged from 8 to 12 for any given trait. Phenotypic variation explained by these QTL ranged from 1.7 to 29.5%. Both parental lines contributed favorable alleles influencing yield. *QHgt.ua-2D* and *QHd.ua-2D* explained the most variance for any given trait and co-localized at a marker for photoperiod sensitivity (*Ppd-D1*). *QTw.ua-5D* accounted for the largest amount of phenotypic variance in test weight (6.7%). Pleiotropic effects occurred where *QTw.ua-6B.2* was associated with the favorable allele from P26R61 for taller plant height and *QYld.ua-5B*, which explained the most variance in grain yield, was associated with the favorable allele from AGS 2000 for early heading. Since early heading date is essential to wheat cultivars adaptably to a broad range of environment, this region could have a positive influence on the improvement of soft red winter wheat in this region. Higher yielding lines accumulated more favorable alleles for yield QTL when compared to low yielding lines. This shows that with small effect QTL the accumulation of favorable alleles is important to increased productivity and this can be accomplished either through a target QTL marker assisted selection program or through genomic selection.

Spanning 11 chromosomes, 14 QTL cluster regions were detected with as many as seven developmental stage NDVI QTL co-localizing together. All seven NDVI QTL clustered on chromosome 4DL with phenotypic variance explained ranging from 4.4 to 10.7%. Interestingly, when clustering together a single parental line contributed all favorable alleles with the exception

of chromosome 6A where AGS 2000 contributed the favorable alleles for all NDVI QTL and P26R61 contributed the allele for vegetative biomass. Development stages showed low to moderate correlation with yield and biomass at maturity. When analyzed as a single stressed environment, Fay14 showed higher correlations with yield and biomass at maturity which is consistent with previous research.

Overall, the analysis of the P26R61/AGS2000 mapping population identified genomic regions that are potential target regions for marker assisted selection for cultivar improvement in the southeastern United States.